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Agriculture Development Fund

FINAL REPORT

HIGH QUALITY STANDARDS FOR RAW AND PROCESSED HERBS, A PREREQUISITE FOR STRONG HERB INDUSTRY IN SASKATCHEWAN

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FINAL REPORT

High quality standards for raw and processed herbs, a prerequisite for strong herb industry in Saskatchewan

ADF Project No. 96000233

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February 2000

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A. Abstract

The general objective of this 3 year project was to continue providing leadership to the herb research and industry development in Saskatchewan with emphasis on evaluating herb quality in research and service capacity. Specifically, the objectives were to assist herb industry to become a supplier of high quality botanicals in crude and processed form, and to identify new products (bioactive compounds) and processing technologies with good potential for commercialization.

All Saskatchewan grown medicinal species analyzed to date were found to be of marketable quality. The quality of dominant species expressed as % of active compounds is: *Echinacea angustifolia*, echinacoside up to 1.8% and cynarin up to 0.32%; feverfew, parthenolide up to 0.96% in leaves and flowers, and up to 1.4% in flowers; milk thistle, up to 2.8% total silymarin in seed; St. John's Wort, up to 0.35% total hypericins and up to 4.0% total flavonoids in flowering tops; valerian up to 2.5% valerenic acids in root. However, a variability in phytomedicinal quality of herbs between the production sites and within a single site was found to be considerable. More efforts need to be directed toward advancing knowledge required for the production of herbs of consistent high quality. Optimal time for harvesting *Echinacea angustifolia* root and flowering tops of St. John's Wort were also established.

The SHRP services provided to herb industry: (1) analysis on request and on a cost recovery basis of 314 samples for medicinal constituents to growers, start-up herb processing companies and research companies; (2) extension activities included over 1000 calls, 190 information mail-outs and over 100 electronic mail-outs, 128 office visits, over 30 tours of Herb Resource and Demonstration Garden, and 27 sample diagnostics. The SHRP engaged in several new collaborative projects addressing quality of medicinal plant production from seed to harvest, and a discovery of two new biological activities in extracts of wild Saskatchewan plants.

B. Executive Summary

This is the final report for the ADF Project #96000233 which commenced in May 1997. General objective of the project was for the Saskatchewan Herb Research Program (SHRP) to continue to provide leadership in herb research and industry development with emphasis on determining quality and identifying new products and processing technologies with potential for commercialization for the Saskatchewan grown herbs; also to assist herb industry in the province to become a supplier of high quality botanicals in crude and processed form. Specific objectives were: (1) to start providing analytical services to the herb industry on the cost recovery basis; (2) to continue updating the Saskatchewan Herb Database; (3) to continue research on Saskatchewan indigenous plants as source of bioactive compounds; (4) to establish communication and, if possible, work together with producer and industry organizations, research agencies, governments, interest groups and networks with common interest in herbs and use of herbs in the emerging field of nutraceuticals. All objectives but the objective (2) were met. In late 1998, the Saskatchewan Herb and Spice Association (SHSA) received funding to prepare 2nd expanded edition of the Herb and Spice Growers Manual, which eliminated the need for updating the Saskatchewan Herb Database. The SHRP has undertaken broader research activities instead.

The quality assessment (including method development) of the following herbs, in alphabetical order, were featured in this report: *Acorus calamus* (calamus root), *Astragalus membranaceous* (Chinese milkvetch), *Calendula officinalis* (garden marigold), *Echinacea angustifolia* (narrow-leaved purple coneflower), *Echinacea pallida* (pale purple coneflower), *Echinacea purpurea* (purple coneflower), *Glycyrrhiza glabra* (common licorice), *Glycyrrhiza lepidota* (wild licorice), *Glycyrrhiza uralensis* (Chinese licorice), *Hypericum perforatum* (St. John's wort), *Matricaria recutita* (German chamomile), *Panax quinquefolius* (American ginseng), *Polygala senega* (senega), *Silybum marianum* (milk thistle), *Tanacetum parthenium* (feverfew), *Urtica dioica* (stinging nettle), and *Valeriana officinalis* (valerian). All Saskatchewan grown medicinal plant species analyzed to date were found to be of marketable quality. However, a considerable variability in phytomedicinal quality of herbs, particularly *E. angustifolia*, was evident. Consequently, it is recommended that more efforts be directed toward advancing knowledge required for the production of herbs of consistent high quality.

Echinacea, grown on 150-200 acres across Saskatchewan, emerged as a major medicinal root crop over the last 3 years. Phytomedicinal quality of various echinacea plant parts and the distribution of marker compounds through the root system were undertaken. Root of *E. angustifolia* and root and flowers of *E. purpurea* were found to contain the highest content of phenolic marker compounds (echinacoside in *E. angustifolia* and chicoric acid in *E. purpurea*), presently recognized by industry as quality indicators. The fine side root branches and root

crowns were found to contain the highest content, while the main body of tap roots contained the lowest content of phenolic markers, echinacoside and cynarin. Study on optimizing harvest time for echinacea root from the phytomedicinal quality perspective was also conducted. The content of natural compounds considered to be responsible for the immunostimulatory activity of echinacea such as phenolics, alkylamides and polysaccharides, were determined in roots collected in spring, mid-summer and fall. Echinacoside content was significantly higher in 2-year old plants than 3-year old plants at all three harvest times, for example 1.29 % in 2-year old vs. 0.34% in 3-year old (fall harvest). Total alkylamides content was higher in 2-year old plants than 3-year old plants and tended to decrease from spring to fall irrespective of plant age. Dominant compound was dodeca-2E, 4E, 8Z, 10E/Z-tetraenoic acid isobutylamide. However, polysaccharide content was significantly higher in 3-year old plants than 2-year old plants and was significantly higher in fall than spring and summer. This study also revealed great plant to plant variability which was responsible for inconsistency of echinacea root quality.

In 1998 the SHRP developed a chromatographic method for verification of echinacea species using seed, which has proven to largely reduced the risk of purchasing mislabeled seed and has also provided a great marketing tool for Saskatchewan echinacea seed on national and international markets. A preliminary study on alternative uses of *E. angustifolia* seed, besides its use as a propagative material, revealed that seed contain some of the same alkylamides as root, chlorogenic acid as one of the dominant phenolics, and high content of digestible fiber. These data suggested that exploration of a range of potential nutraceutical applications of seed is a worthwhile effort.

In-depth study on the effect of harvest time and plant part on the phytomedicinal quality of St. John's wort was performed. The flowering tops followed by the upper most leaves contained the highest concentration of hypericins and flavonoids when harvested in late June, 0.35 % and 4.0%, respectively. The hypericins content declined by more than 90% between late June and end of August. The content of selected flavonoids showed a similar declining trend from early July onward. Two varieties, Anthos and Elixir ™, were found superior in both plant yield and plant quality.

The SHRP maintained its involvement in several collaborative research projects such as: "Northern Vigour™" on 6 medicinal and aromatic plant species, Herb Quality Management component of the AFIF project on medicinals, and screening studies of wild Saskatchewan plants for immunostimulatory activities. It has also commenced collaborative studies on antioxidative and anticancer activities from wild Saskatchewan plants, and one interprovincial project with Alberta and Manitoba, which addresses a need to identify superior seed sources of *E.*

angustifolia. A novel immunostimulatory and an antioxidative activity have been identified for two wild Saskatchewan plants, with good opportunity for commercialization.

The SHRP assisted members of the SHSA with multitude of their needs in production, processing and marketing of herbs. The extension activities included over 1000 calls, 190 information mail-outs and over 100 electronic mail-outs, 128 office visits, over 30 tours of Herb Resource and Demonstration Garden, and 27 sample diagnostics. Direct assistance to several Saskatchewan start-up companies such as Wise Owl Herbs Co. Ltd., Maple Ridge Farm Inc., Coulee Pickings Ltd., Blue Hills Herb Farm, Inc. and other groups and organizations such as Norsask Botanicals, Inc., Northern Lights Herbs, Inc. and Conservation Learning Centre in Prince Albert, was provided. Information pertaining to herb quality, herb production, and novel uses of herbs were delivered at over 25 presentations including annual meetings of the SHSA, and articles were featured in the SHSA Newsletter. Productive communication and information exchange was achieved with Saskatchewan Nutraceutical Network, Native Plant Society of Saskatchewan, and Saskatchewan Food Processing Association.

On herb industry demand in summer 1997, SHRP started providing analytical services on a cost recovery basis. Since then, the 328 industry samples were analyzed, 75% of them were related to echinacea. The highest number of requests was received for echinacea root quality assessment, followed by species verification of seed and root, and analysis of echinacea herbal preparations. The percentage of misidentified seed tested in our laboratory decreased from about 20% in early 1998 to almost nil in late 1999. A growing supply of *E. angustifolia* seed of reliable authenticity from Saskatchewan has provided credibility and competitiveness to the Saskatchewan echinacea industry. The remaining analyses provided by SHRP included essential oils from cilantro, dill weed, and caraway, analysis of feverfew plant and commercial preparations, St. John's wort plant and preparations, milk thistle seed, valerian root, burdock root etc. We have experienced a steady increase in demand for our analytical services.

Over the past 3 years the average growth rate of the herb industry in North America continued to be high, over 20%, and myriad of new activities in both industry and academia has taken place. At the same time, given increase in herb production and processing capabilities and emergence of over 10 new companies, Saskatchewan herb industry has reached a new higher level and is poised to continue its growth. Medicinal plants are new specialty crops for Saskatchewan with promising future. It is believed that the leadership and technical support of SHRP has played an invaluable role in Saskatchewan herb industry growth and competitiveness, particularly in the area of high quality standards.

C. Technical Report

OBJECTIVE 1: to continue developing expertise in quality evaluation of herbs and providing analytical services to herb industry on the cost recovery basis.

Achievements

1. Echinacea Root

Three main areas of activities were: (1a) species verification of three commercial *Echinacea* spp. using the chromatographic methods developed in 1996; (1b) evaluation of phytomedicinal quality of echinacea using current industry standards and establishing the distribution of phenolic marker compounds in plant material collected in the wild, and grown under field and greenhouse conditions; (1c) determination of the effect of harvest time on phytomedicinal quality; (1d) and comparative random testing of commercial preparations of echinacea available on the market.

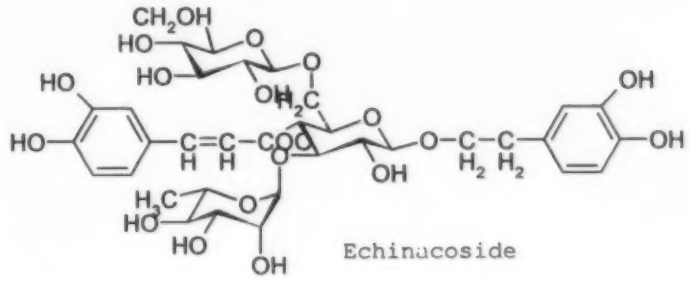
1a) Species Verification

One and first quality parameter in the field of medicinal plants is proper plant identification. Species identity is typically determined by a botanist on the basis of morphological characteristics of a plant, however, in some instances it could also be determined chemically. Chemical structure of dominant phenolic marker compounds, that could be used as taxonomic markers for three commercial *Echinacea* species are presented in Figure 1. Echinacoside and cynarin are characteristic for *E. angustifolia*, echinacoside for *E. pallida*, and chicoric acid for *E. purpurea*. An HPLC (high performance liquid chromatography) fingerprint of hydrophilic root extracts of Echinacea spp. of commerce is presented in Figure 2. The authentication of *Echinacea* spp. is, however, commonly conducted by chemical fingerprinting of the lipophilic root extract (Figure 3), which is a reliable method for distinction between between *E. angustifolia* and *E. pallida* using lipophilic fraction of the root. The same technique was found only partially useful in species verification of two other species of the genus *Echinacea*, *E. paradoxa* and *E. simulata*. Based on the limited number of tests, their fingerprints resembled to a great extent *E. pallida* profile, and were difficult to distinguish from each other. Further studies are required to determine the feasibility of using this chemical test for species verification of other species in the genus *Echinacea* but the three species of commerce.

Species verification of echinacea root has become one of the most frequently requested analysis by the growers and was instrumental in eliminating several suppliers of unauthentic seed in

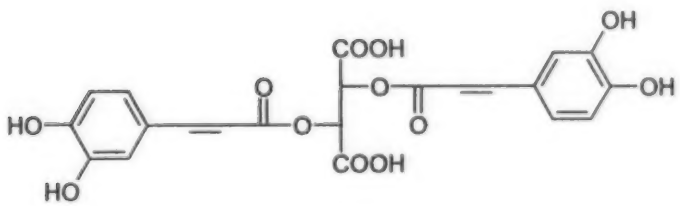
the province. This chromatographic method for species verification could be applied to a sample of ground root, a liquid extract, or a pill of a commercial echinacea-based product from a local health food store with equal accuracy. This analytical capability is a great tool that is being frequently used by the industry. It could also prove indispensable in the market surveillance of echinacea botanical preparations by the regulatory authorities.

Figure 1. Chemical Structures of Marker Compounds in Commercial Echinacea Species



Echinacoside

Cynarin



Chicoric Acid

Figure 2. HPLC Fingerprints of Hydrophilic Extracts of Echinacea Root

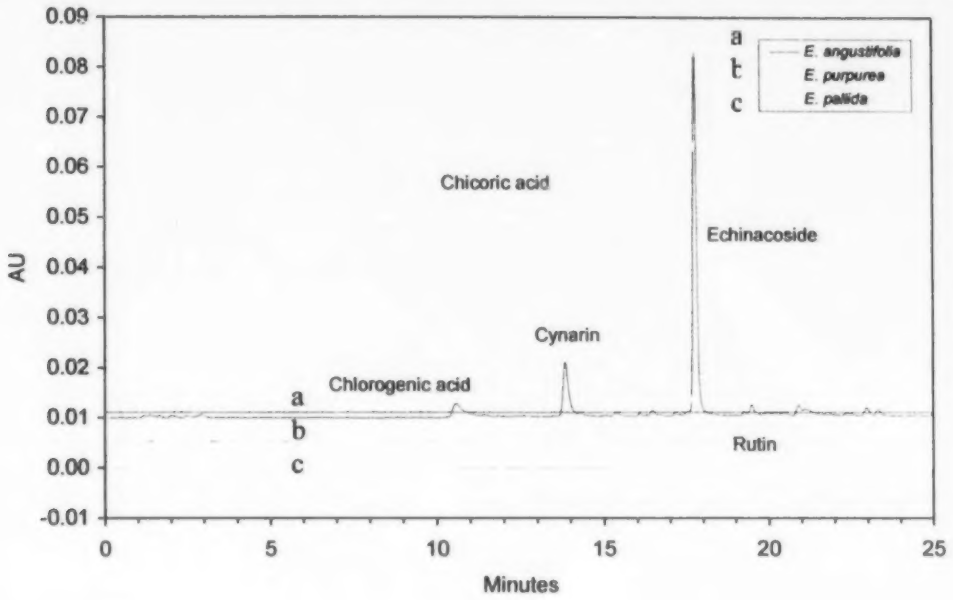
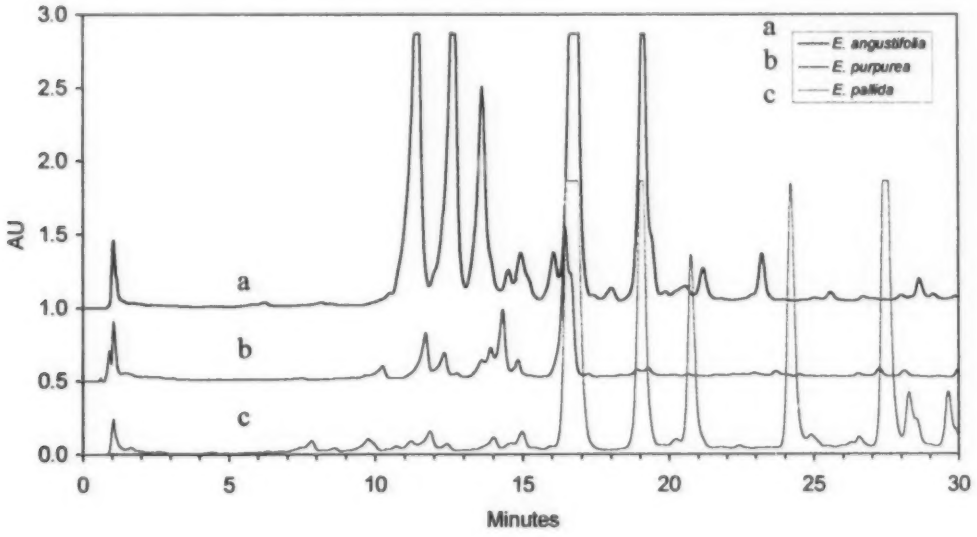


Figure 3. HPLC Fingerprints of Lipophilic Extracts of Echinacea Root



1b) Quality Analysis of Root

Three main requirements for medicinals and botanical preparations are to be safe (of no toxicity), efficacious (exhibit clinically recognized beneficial effect in human body) and of high quality. The main quality parameter that Herb Research Program is addressing through its research is the content of physiologically "active" compounds. In cases when "actives" are unknown, the content of marker compounds is determined instead. Marker compounds are natural compounds characteristic for a given plant species, accepted by industry as useful compounds for monitoring batch to batch consistency and for other quality control purposes, but not necessarily physiologically active. Over the past three years we were involved in determining phytomedicinal quality of echinacea root, flowers and leaves in plant material collected in the wild and grown under field and greenhouse conditions.

The distribution of phenolic marker compounds in roots of *E. angustifolia*, *E. pallida*, *E. purpurea* and *E. simulata* (species unconfirmed) collected either in the wild (Saskatchewan and Montana) or grown under field and greenhouse conditions, is shown in Figures 4, 5, 6 and 7. Interestingly, the root of *E. simulata* contained the highest content of echinacoside, followed by Saskatchewan wild and Montana wild *E. angustifolia* (Fig. 4). The *E. simulata* root also contained about 0.07% of chicoric acid, which is a characteristic marker for *E. purpurea*. Cynarin was found present only in *E. angustifolia*, which confirmed potential use of cynarin as a chemotaxonomical marker for *E. angustifolia* (Fig. 5). The 2-year old *E.purpurea* contained 0.225% of chicoric acid (Fig.6). Chlorogenic acid and rutin were the minor components beside the chlorogenic acid content in the root of *E. purpurea* (0.065%, Fig. 7).

Root of *E. angustifolia* contained the highest content of echinacoside, followed by flowers, leaves and stems (Fig. 8). In our 1998 study we found that irrespective of root age, the young side root branches, root hair and root crown consistently contained higher content of echinacoside and cynarin, than the main body of *E. angustifolia* tap root (Figure 9). This non uniform distribution of phenolic compounds throughout the root system suggests that harvesting has direct implications not only on root yield, but quality as well. It seemed important o harvest roots as deeply as possible to recover young rootlets as completely as possible, and to also retain root crown in its entirety.

A preliminary study on the effect of aster yellows, a disease caused by the aster yellows phytoplasma, in the first year of symptoms development on quality of *E. angustifolia* root was conducted in 1998/99. Interestingly, no correlation was found between the content of phenolic compounds and the extent of the disease damage. Although this disease has a tremendous impact on echinacea seed production, it apparently does not influence quality in first year of symptom development. It would be of interest to further investigate the effect of aster yellows on root quality.

Figure 4. Echinacoside Content in Root of Echinacea Species

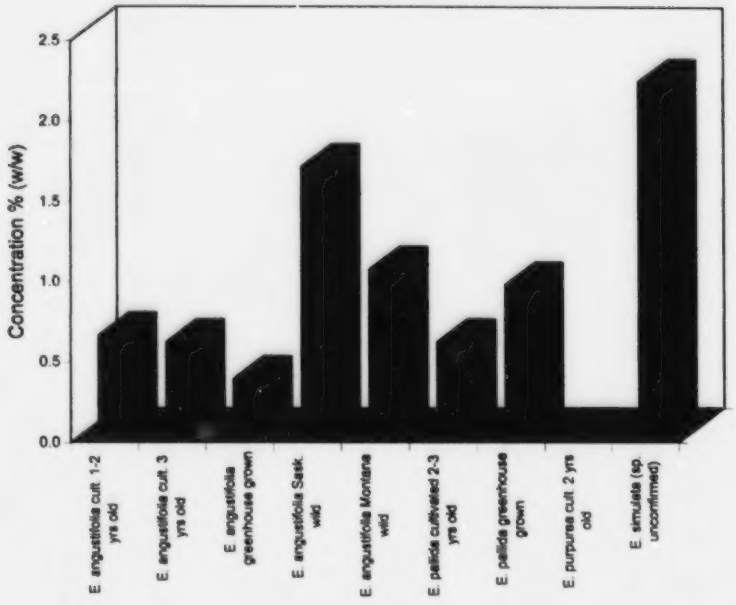


Figure 5. Cynarin Content in Root of Echinacea Species

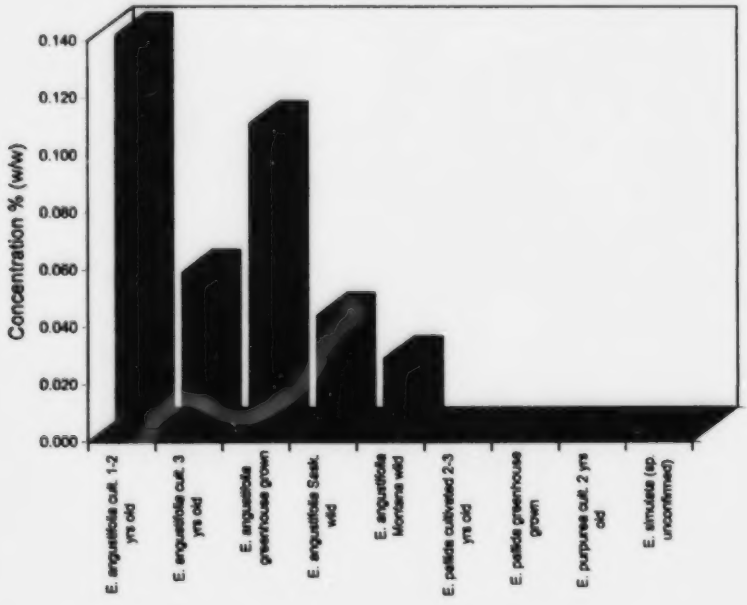


Figure 6. Chicoric Acid Content in Root of Echinacea Species

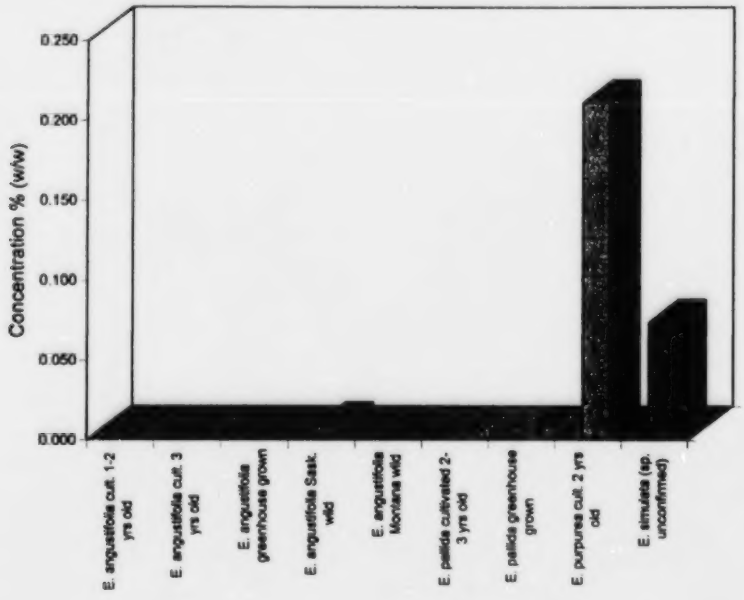


Figure 7. Chlorogenic Acid Content in Root of Echinacea Species

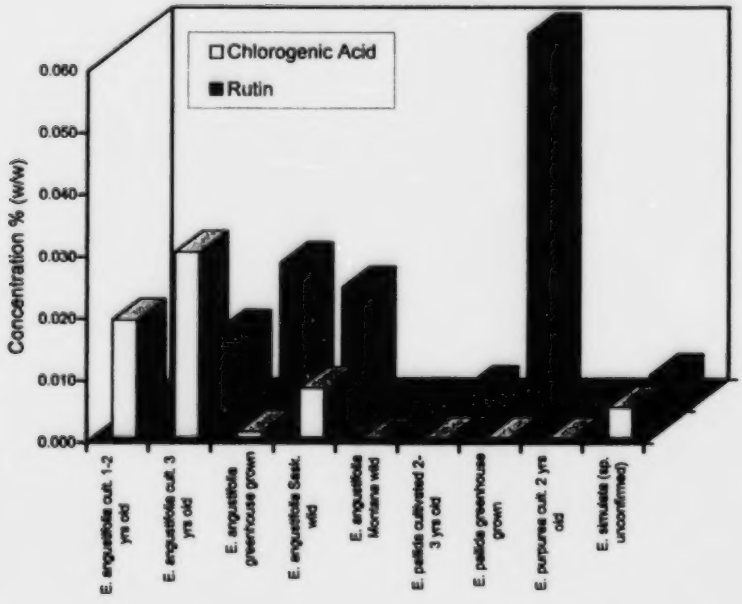


Figure 8. Content of Marker Compounds in Various Plant Parts of *Echinacea angustifolia*

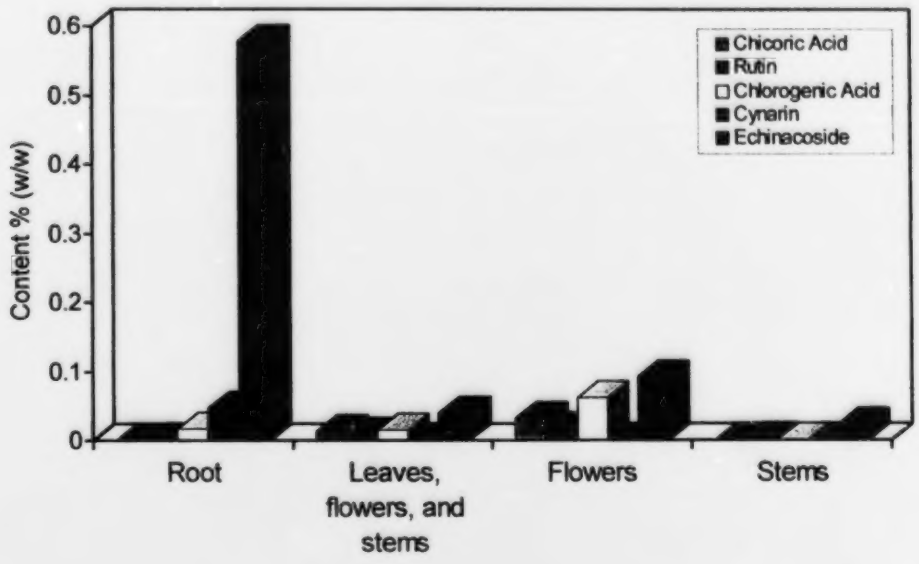
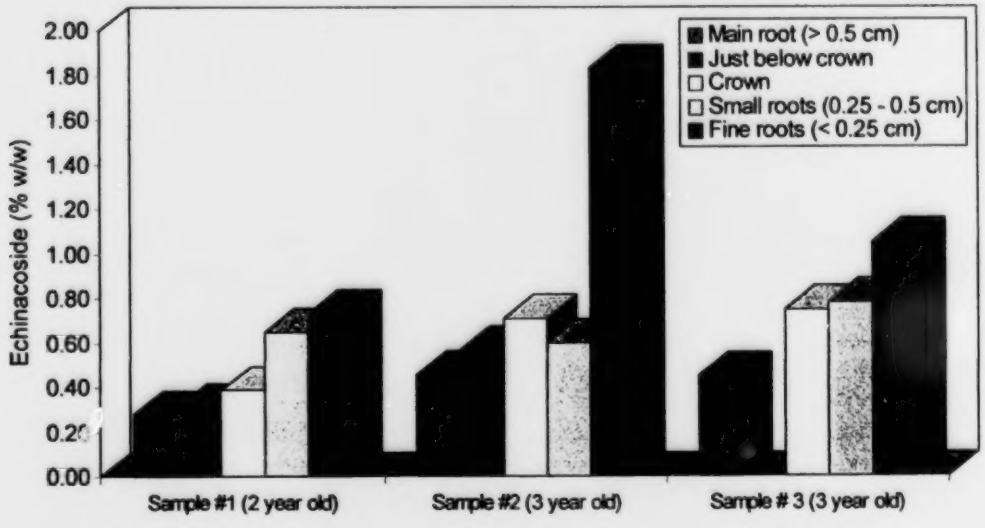


Figure 9. Distribution of Echinacoside in Echinacea Root Harvested in 1998



1c) Effect of Harvest Time on Quality

In 1998 in collaboration with echinacea growers from Saskatchewan and SHSA we made a first attempt to establish relationship between the content of specific phenolic compounds, recognized by industry as echinacea quality indicators, and harvest time. Analysis of *E. angustifolia* root for the content of echinacoside and cynarin, *E. pallida* root for the content of echinacoside, and *E. purpurea* root for the content of chicoric acid, in spring, summer and fall, produced inconclusive results. Contrary to expectations, no trend in accumulation of phenolics from spring to fall could be observed in any of three species tested. We came to realize that by sourcing the material from growers across Saskatchewan we evidently introduced a large number of variables from seed source, soil type, soil fertility and agronomic management practices, harvesting method and post-harvest handling.

We repeated this study in 1999 using root from a single production site, echinacea plots of SIDC in Outlook. The entire roots of three 2-year old and three 3-year old *E. angustifolia*, and 3 root samples of 4-year old *E. purpurea* , were collected in spring (May 27), summer (July 28) and fall (October 27). Root samples were cleaned after harvest and dried under forced air ventilation at 35°C to moisture content of 9-10%. Samples were stored at 10°C prior to analysis for the content of phenolic markers, alkylamides and polysaccharides during November and December.

The results of phytochemical testing showed great plant to plant variability. The content of echinacoside and cynarin in *E. angustifolia* was found to range from 0.18 to 1.75 % and 0.06 to 0.48%, respectively (Figures 10 and 11). Echinacoside content was significantly higher in 2-year old plants than 3-year old plants at all three sampling times (Figure 12). Average echinacoside contents for 2-year old echinacea were 0.94% for spring harvest, 1.28% for summer harvest and 1.29 for fall harvest. Average echinacoside contents for 3-year old echinacea were 0.30% for spring harvest, 0.16% for summer harvest and 0.34 for fall harvest. Relative content of total alkylamides had a tendency to decrease from spring to fall irrespective of plant age, and was higher in 2-year old plants than in 3-year old plants (Table 1). Polysaccharide content was found to be significantly higher in 3-year old plants, than 2-year old plants, also significantly higher in fall than spring and summer (Figure 13).

The data obtained up to date suggest that echinacea industry has yet many challenges ahead. From the quality perspective, the most obvious problem is a great plant to plant variability causing an inconsistency in quality of crude drug.

Figure 10. Effect of Harvest Time on Content of Marker Compounds in Two Year Old *E. angustifolia* Roots (1999 Harvest)

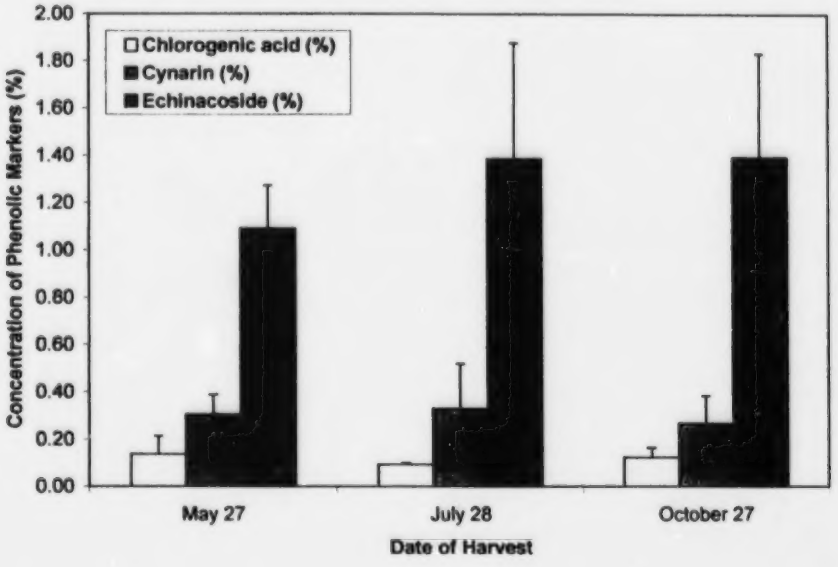


Figure 11. Effect of Harvest Time on Content of Marker Compounds in Three Year Old *E. angustifolia* Roots (1999 Harvest)

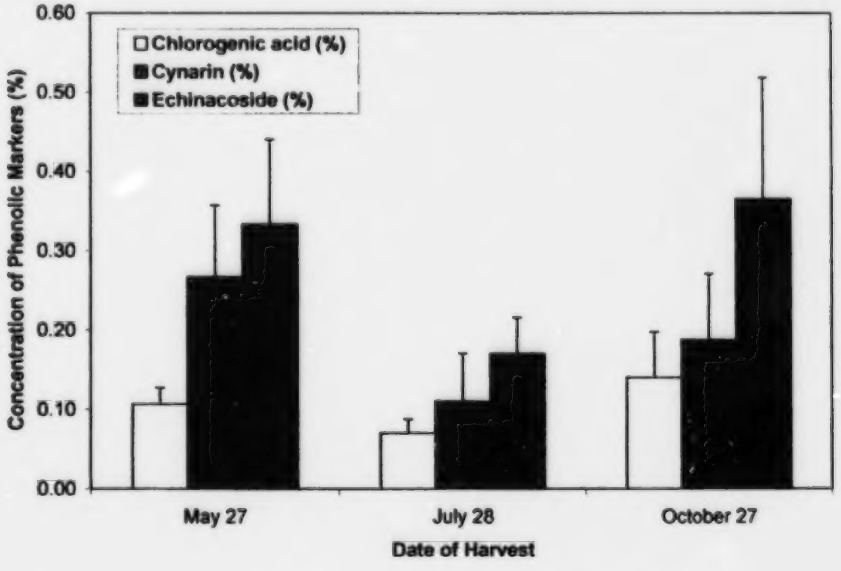


Figure 12. Effect of Root Age on Phenolic Marker Content in *Echinacea angustifolia* (1999 Harvest)

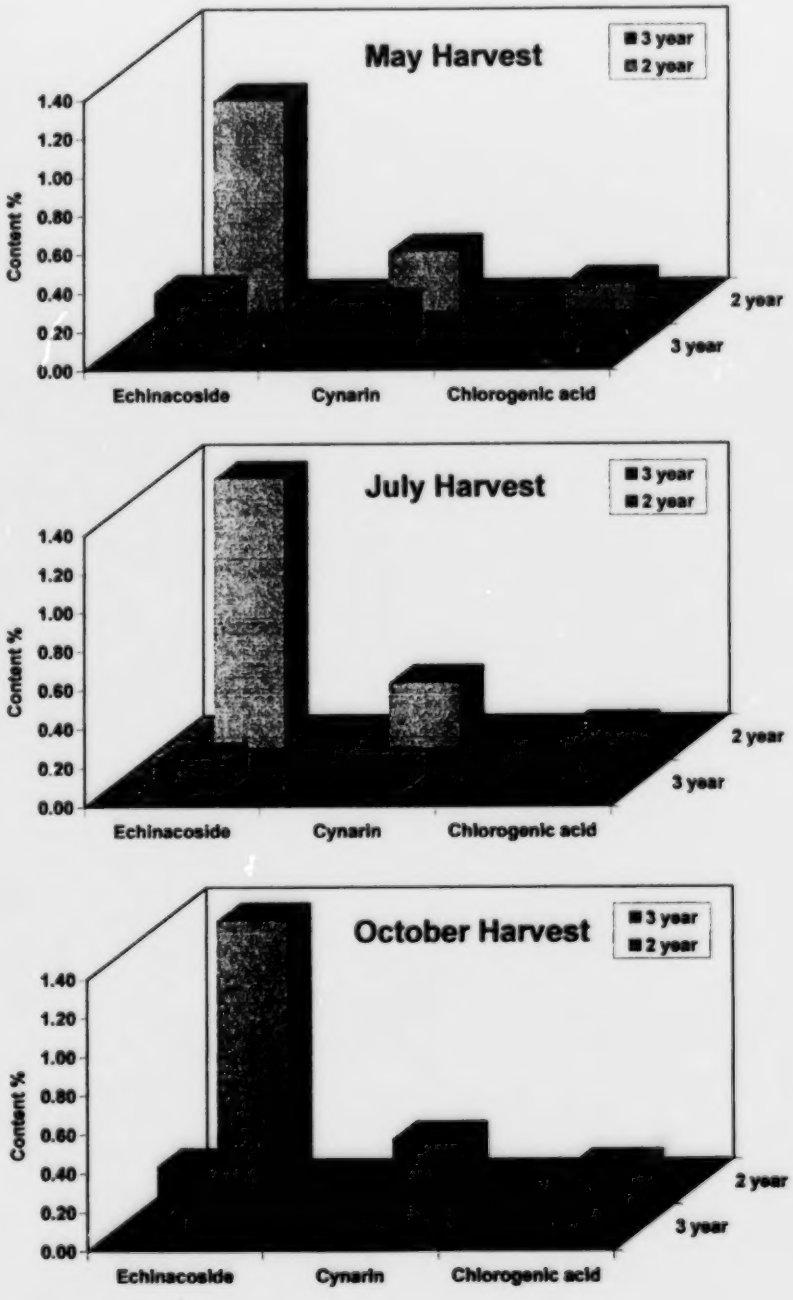
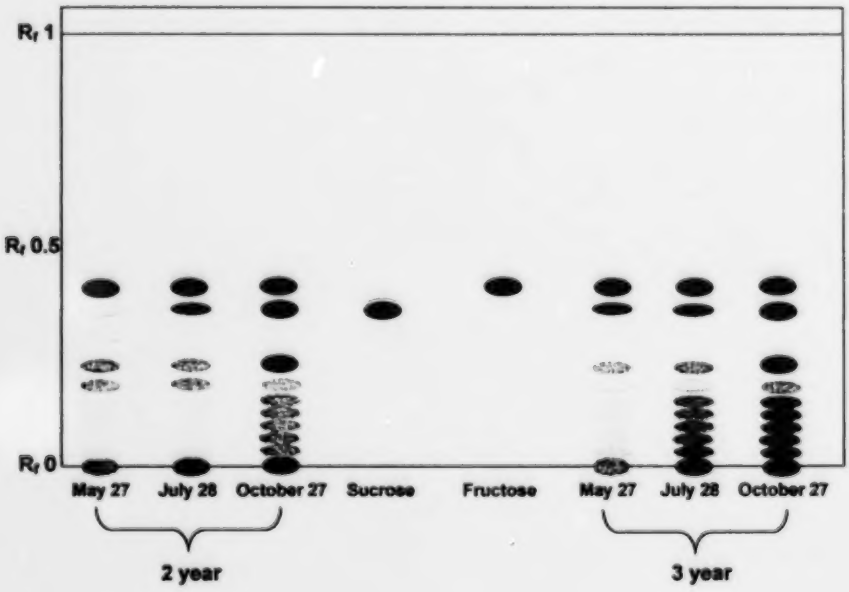


Table 1. Effect of Harvest Time and Age on Relative Content of Alkylamides in *Echinacea angustifolia* Root[1] (1999 Harvest)

	Peak 1 (9.3 min)	Peak 2 (10.9 min)	Peak 3 (12.0 min)	Peak 4 (16.0 min)[2]	Peak 5 (19.0 min)	Peak 6 (21.6 min)	Peak 7 (23.9 min)	Total Area
2 year								
May	10.7	14.5	6.6	30.2	12.6	1.1	1.2	76.9
July	3.2	4.8	3.7	17.5	5.0	0.5	0.6	35.3
October	5.1	8.3	5.6	15.2	6.2	0.6	0.7	41.7
3 year								
May	4.4	7.4	3.0	10.0	3.4	0.1	0.0	28.3
July	3.6	7.2	3.5	13.2	3.4	0.4	0.0	31.3
October	3.3	5.7	2.7	9.8	2.5	0.1	0.0	24.1

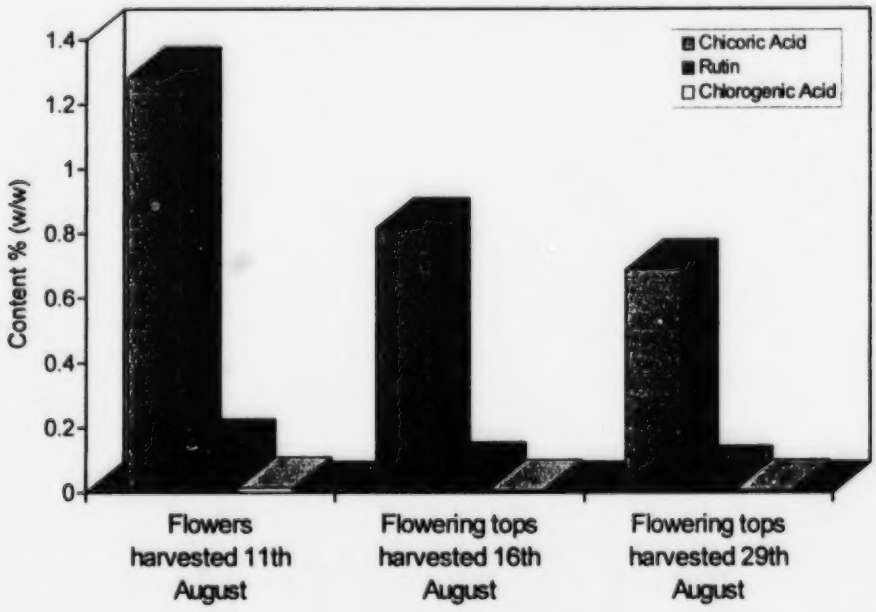
[1] Content of alkylamides (1-7) expressed as peak area units x 10^6 @ 210 nm
[2] Isomers dodeca-2E,4E,8Z,10E-tetraenoic acid isobutylamide and dodeca-2E,4E,8Z,10Z-tetraenoic acid isobutylamide (Bauer and Remiger, 1989)

Figure 13. Effect of Harvest Time on Polysaccharide Content in *Echinacea angustifolia* Roots (1999 Harvest)



E. purpurea was tested for the influence of harvest time on quality of both flowering tops (provided from a grower in British Columbia in 1998) and root (collected at the SIDC plot in Outlook, 1999). With respect to flowering tops, the content of chicoric acid was the highest at the pick of flowering (1.23%, August 11), but decreased 40-50% when harvest was postponed for 1-2 weeks (0.63%, Figure 14). The content of chicoric acid in root was less dependant on harvest time than in flowering tops. It was the highest in root harvested in fall (0.7%), then decreased to 0.48% in root harvested in late May (Figure 15). This study indicate that in order to ensure high quality plant material, it is extremely important to optimize harvesting time.

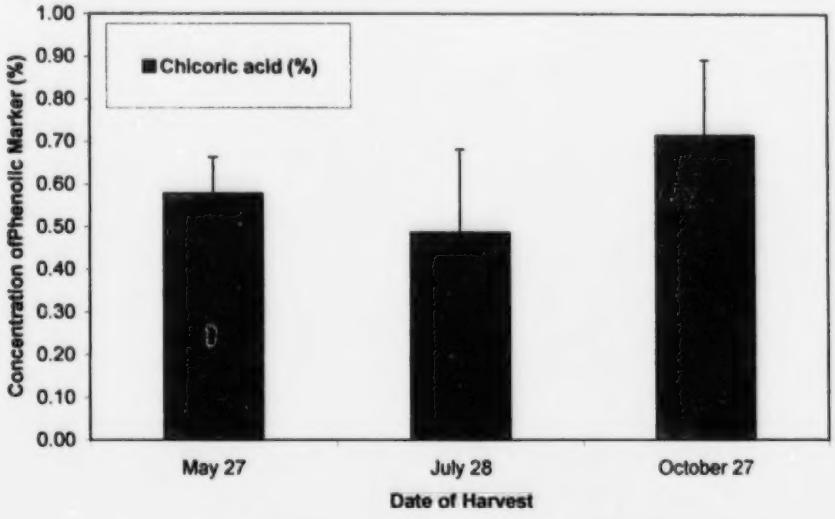
Figure 14. Effect of Harvest Date on Content of Marker Compounds in *Echinacea purpurea* (1998 Harvest)



1d) Quality of Commercial Preparations

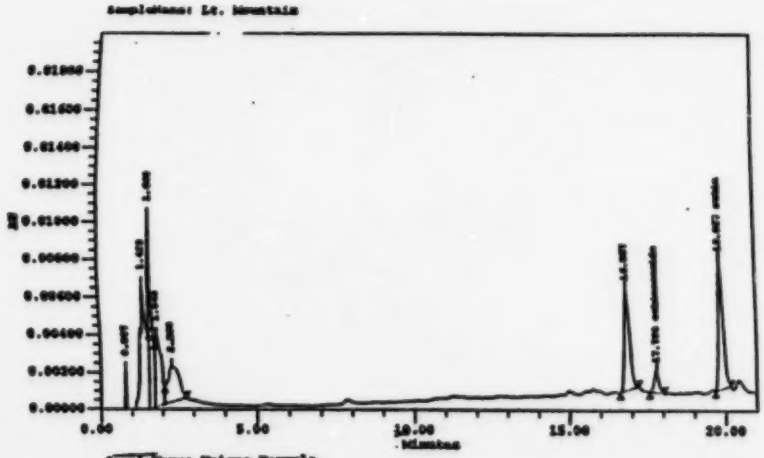
Considering that polysaccharides are believed to be at least partially responsible for the immunostimulating activity of echinacea products, a set of 9 commercial echinacea tinctures was tested for the presence of these compounds by thin layer chromatography (TLC; Figure 16, lanes 1-9). Several sugar standards (maltose, rhamnose, sucrose, xylose) and two types of maltodextrins

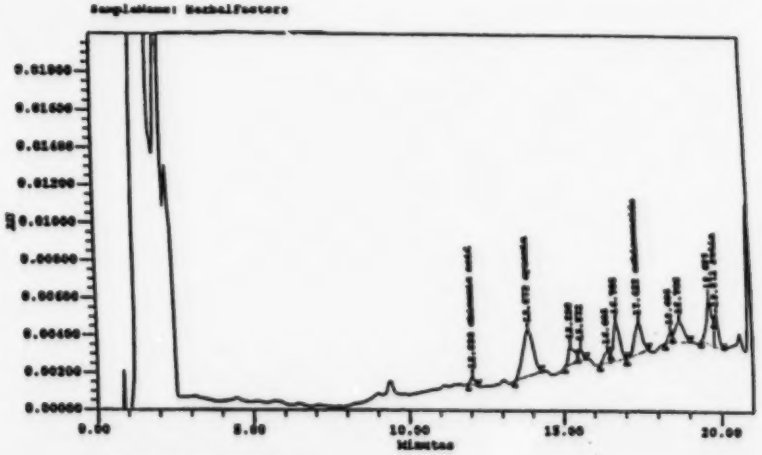
Figure 15. Effect of Harvest Time on Content of Chicoric Acid in Four Year Old *E. purpurea* Roots (1999 Harvest)

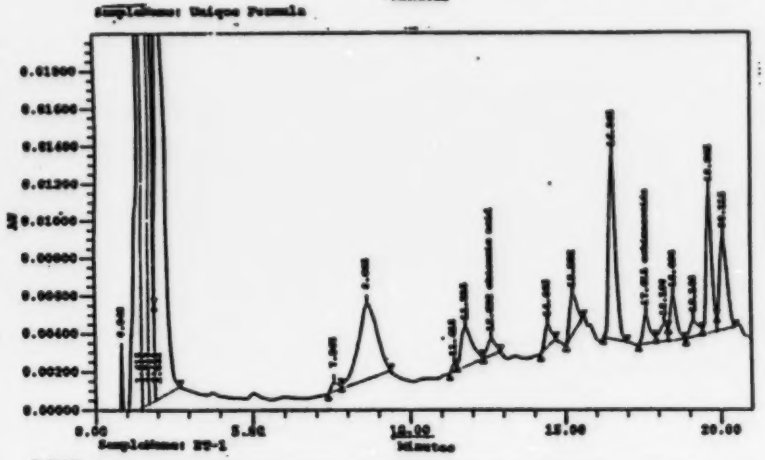


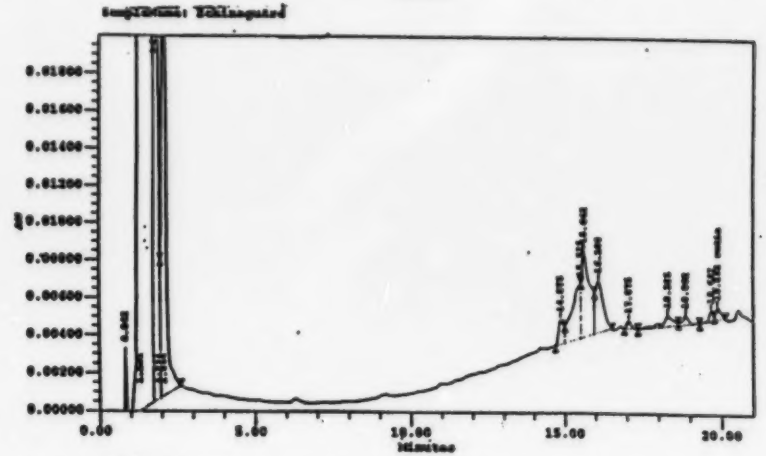
were run as reference standards. The summary of analysis of 6 commercial echinacea tinctures for the content of phenolic marker compounds is presented in a composite Figure 17. A great variation in the profiles of both phenolics and carbohydrates in the preparations tested suggested a wide and unpredictable range of products quality on the market. It was encouraging and worth noting that chicoric acid, a marker for *E. purpurea* was not found in *E. angustifolia*, and that echinacoside, a marker for *E. angustifolia*, was not found in *E. purpurea*. An overall impression was that all of the products tested have a very low content of phenolic glycosides and quite variable content of carbohydrates.

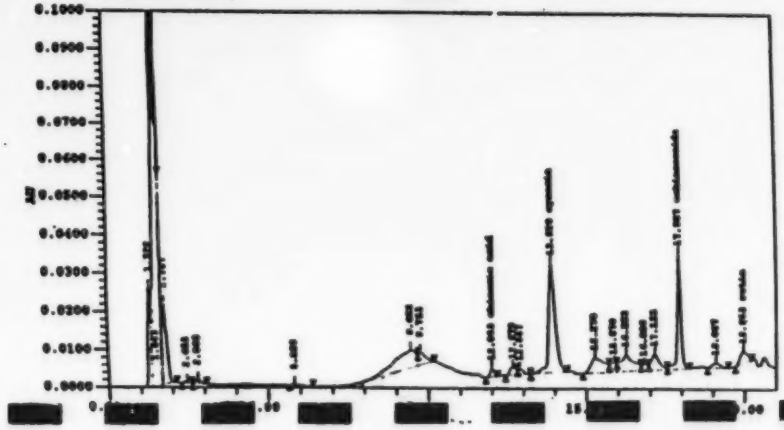
Figure 16. TLC Profile of Carbohydrates in Commercial Echinacea Tinctures

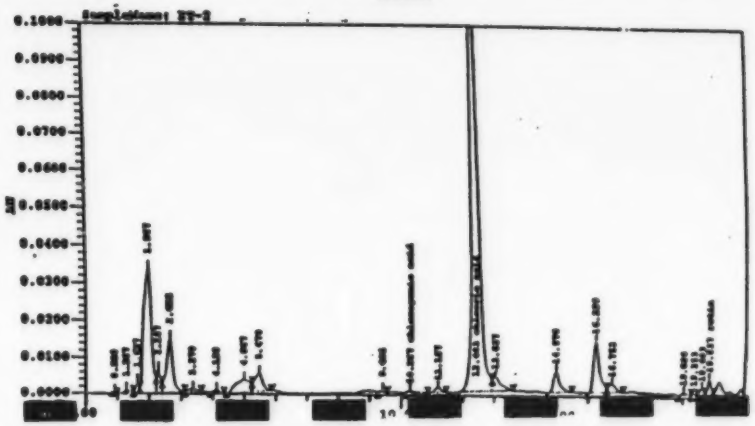

Sample Name: Lt. Mountain
AU
Minutes


Sample Name: Herbal Factors
AU
Minutes


Sample Name: Unique Formula
AU
Minutes


Sample Name: Echinacea
AU
Minutes


Sample Name: ZT-1
AU


Sample Name: ZT-2
AU

2. Echinacea Seed

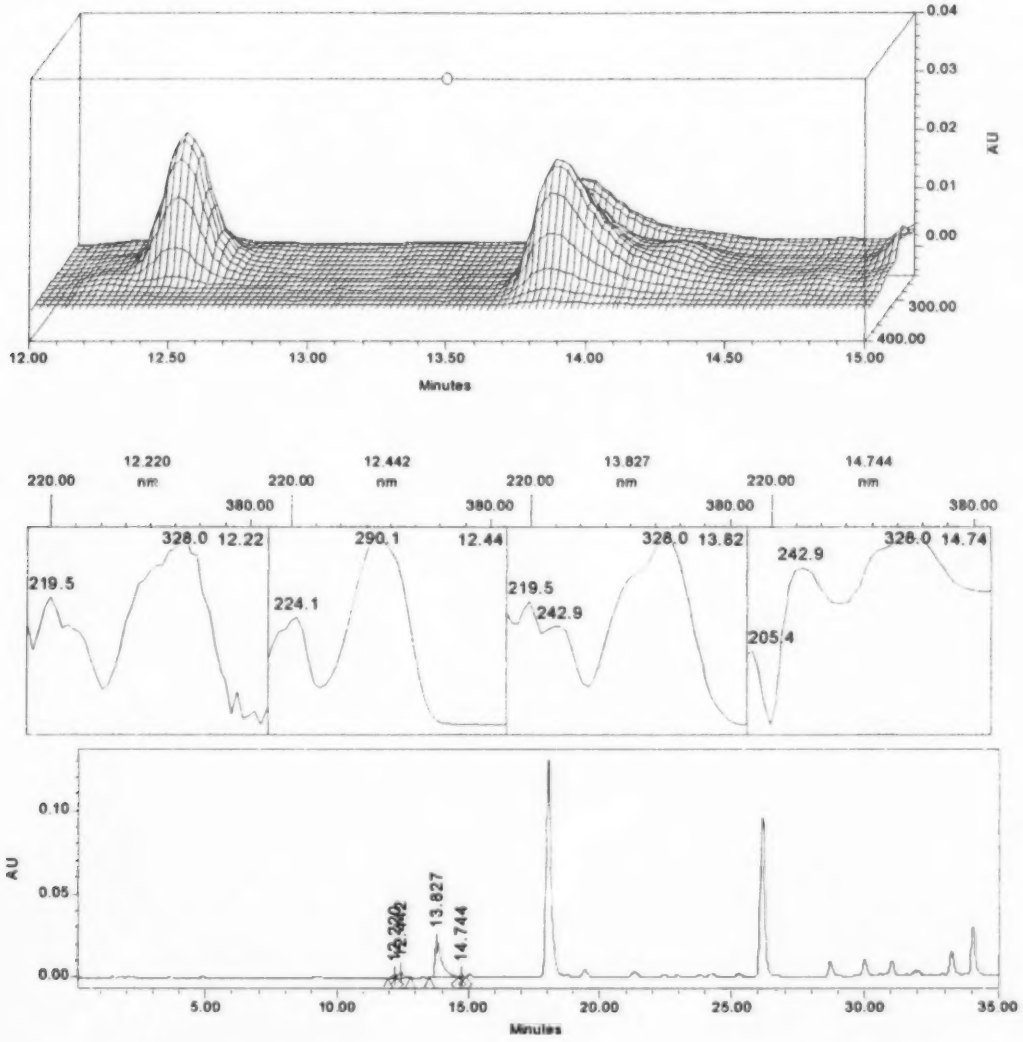
Two main activities concerning echinacea seed were species authentication (emphasis was on *E. angustifolia*), and a preliminary study on alternative seed applications besides its primary application as a propagative material.

2a) Species Verification

It has been recognized by the Herb Research Program has recognized that in early stages of commercial cultivation of echinacea in Saskatchewan one of the limitation facing the industry was the lack of information on authenticity of propagative material, particularly seed. Several herb growers in Saskatchewan have suffered financial losses due to purchasing mislabeled seed of echinacea. That became apparent upon chemical fingerprinting of 2-3 years old roots, long time after the seed was purchased. Consequently, we first made efforts to characterize seed of *Echinacea* species for authenticity on macro- and micro-scale.

The achenes (fruits) of therapeutically used *Echinacea purpurea* could be differentiated structurally (by seed shape and anatomy) from achenes of *E. angustifolia* and *E. pallida*. However, the highly-priced achenes of latter two species were difficult to differentiate with high degree of certainty based on seed appearance and microstructural details only (tissue and cell organization), even at magnifications of up to 40X using optical microscope. Realizing an immediate need to secure authentic seed of commercially important echinacea species and in effort to clear the confusion on the medicinal plant market, we pursued developing an objective and reliable chemical method. Upon analyzing chloroform and methanolic extracts of echinacea seed from over 30 sources, we were successful in developing a TLC and HPLC chromatographic method for species verification of *E. angustifolia* seed. The test is able to reveal whether seed is of true species, or a cross between the species. A particular marker compound with retention time Rt=13.90 min (Figure 18), whose structure is presently being elucidated, was found to be consistently present in seed of *E. pallida,* and seed of several other *Echinacea* spp., but is absent from *E. angustifolia* seed. The isolation and structure elucidation of this marker compound using various spectroscopic methods (LC-MS and NMR), is currently in progress. According to our results to date, it appears that seed extract, in addition to root extracts of *E. angustifolia*, has a distinct chromatographic profile that could be used for establishing species identification with high certainty. Given the large number of inquiries and requests for echinacea seed authentication by the growers over the last year, it is evident that this test has been well received by the industry. Due to the novelty of the test, it of no surprise that many requests for testing were received from and outside of Saskatchewan.

Figure 18. Chromatographic and Spectroscopic Profiles of *Echinacea pallida* Seed Extract



2b) Alternative Uses

Assuming a plant density of 25,000-30,000 plants /acre, 10 grams of dry seed per plant at an average 30 flowers per plant, and 30% seed loss on cleaning, one acre field of *E. angustifolia* could yield >175 kg/acre of seed in third and fourth year of cultivation. At an average price of $1,000 per kilogram, the seed component of commercial echinacea cultivation could potentially contribute > $100,000 annually thus having a significant impact on the profitability of the operation.

If an interest in *E. angustifolia* as a medicinal crop for Saskatchewan continues to increase at a same rate it had been over the past three years, there is a legitimate concern that echinacea industry will face an oversupply of seed, and consequently a likely price drop. In anticipation of this possibility, Saskatchewan Herb Research Program has initiated a preliminary study on alternative uses for *E. angustifolia* seed from a broad chemical composition perspective. The results of proximate analysis of two seed sources, one originating from cultivation in Saskatchewan (1999), and the other one from the wild in Manitoba (1997), are presented in Table 2. It is evident that seed has high lipid content, 18.5-21.9%, but no unique fatty acid profile of its oil (Table 3). The dominant fatty acids are those commonly occurring in oilseed crops, linoleic (18:2), oleic (18:1), and palmitic (16:0) acids. It is of interest to note that digestible fiber (soluble and insoluble) content is relatively high, which suggests a potential use of seed as a functional food or nutraceutical ingredient. Also, digestible fiber appear to be a dominant carbohydrate component (60-75 %) in echinacea seed. Seed also contains alkylamides, but at a lower level that in the root; interestingly, the same alkylamide compound (dodeca-2E, 4E, 8Z, 10E/Z-tetraenoic acid isobutylamide) was found dominant in both root and seed (Figure 19). Providing alkylamides are partially responsible for the immunostimulating activity of echinacea, seed could be used as a supplemental source of these compounds to root in botanical preparations of echinacea intended to combat flue and cold. We have also found that seed contain 0.3 to 0.6 % essential oil. Phenolic compounds were also found, but with a different profile than in echinacea root, no echinacoside and cynarin, but up to 0.6% of chlorogenic acid (Figure 20); the latter can be considered a phytomedicinal ingredient with moderate antioxidative activity.

Further analyses as well as testing for feasibility of using echinacea seed in various nutraceutical and phytopharmaceutical products are required. Considering that echinacea is being incorporated into a wide variety of products on the mass market, from ointment for skin wounds to chewing gum, lip balm, toothpaste, mouthwash etc., it is worth exploring a range of potential applications based on seed as a source. Besides the products aimed for human consumption, echinacea seed should also be explored as an ingredient in specialty feeds and for other veterinary applications.

Table 2. Proximate Analysis and Total Digestible Fiber Content in *Echinacea angustifolia* Seed

Sample I.D.	Moisture (%)	Lipid (%)	Protein (N x 6.26) (%)	Ash (%)	Total Carbohydrates[1] (%)	Total Digestible Fiber (%)
Cultivated #1	6.67	21.9	34.9	4.08	32.45	24.97
Wild #2	6.20	18.5	27.9	6.27	41.13	28.58

[1] Calculated by weight difference

Table 3. Fatty Acid Profile of *Echinacea angustifolia* Seed Oil

Sample I.D.	C14	C16	C16:1	C18	C18:1	C18:2	C18:3	C20	C20:1	C22	C24
Cultivated #1	0.36	5.09	0.19	2.65	11.45	77.51	1.12	0.45	0.14	0.58	0.18
Wild #2	0.33	5.92	0.25	2.63	15.14	72.57	1.06	0.62	0.16	0.83	0.26

Figure 19. HPLC Profiles of Lipophilic Extracts of *Echinacea angustifolia* Root and Seed

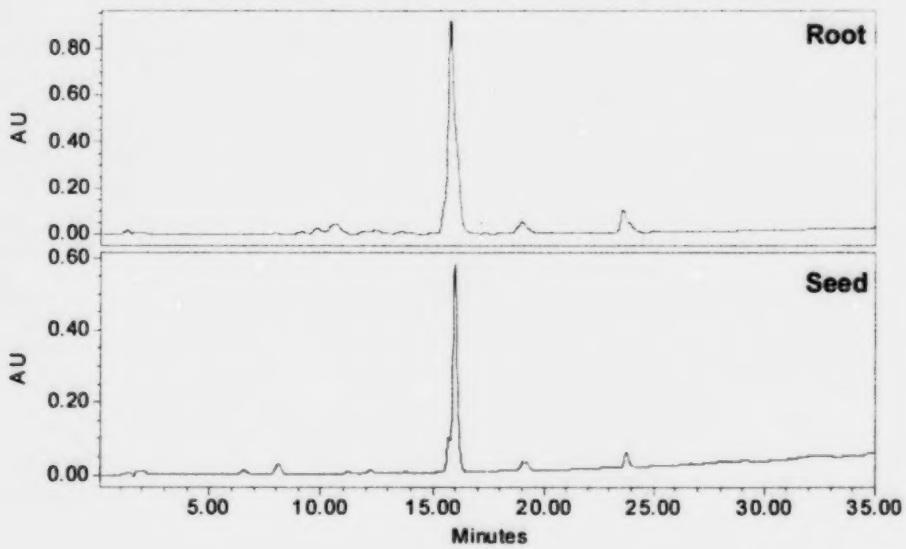
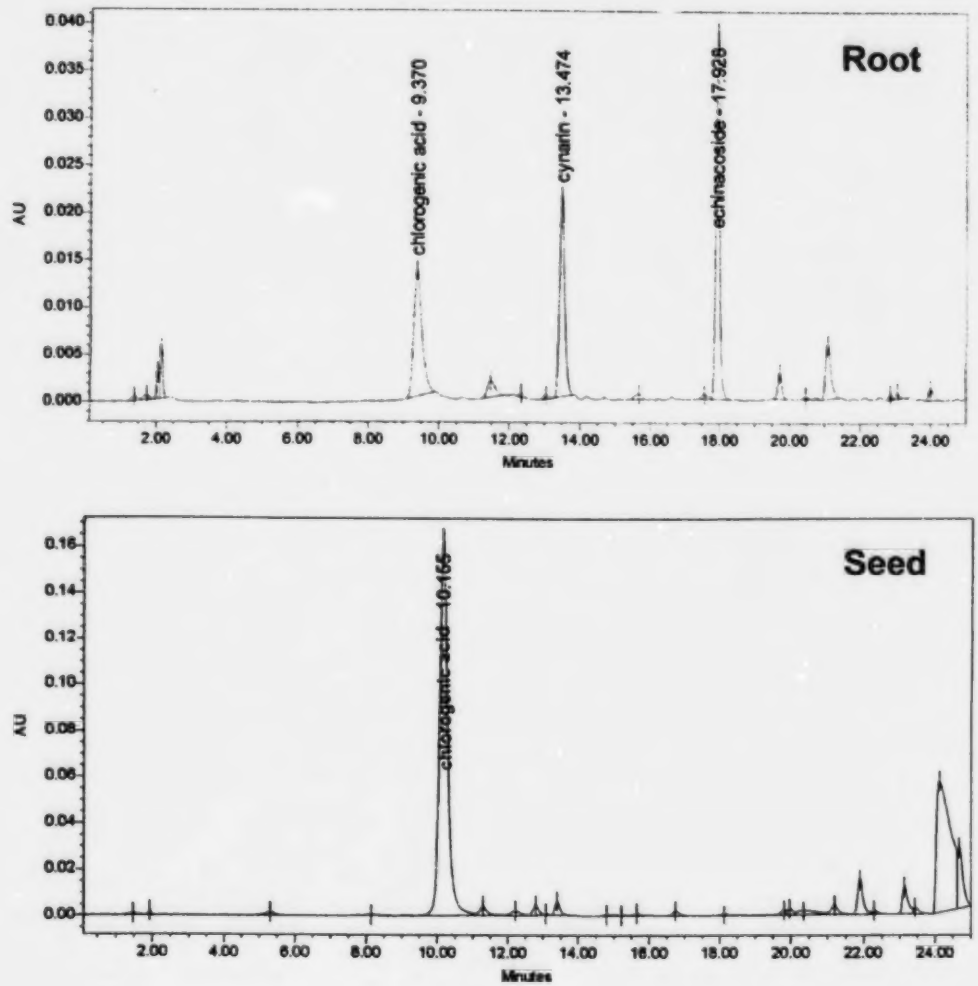


Figure 20. HPLC Profiles of Phenolic Compounds in *Echinacea angustifolia* Root and Seed



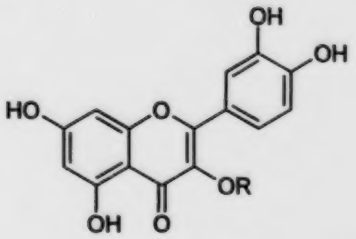
3. Saint John's Wort – Quality Assessment and Effect of Harvest Time on Quality

The St. John's Wort (*Hypericum perforatum*) was recently documented to posses mild antidepressant activity, which caused an explosive increase in demand for the herb in North America. It was an opportune time for interested growers in Saskatchewan to test feasibility of large scale cultivation of St. John's Wort in the province. Since it is a noxious weed in pastures throughout North America, Europe, and Asia, growing Saint John's wort should not present a challenge. Critical factors for competitive commercial cultivation would, however, include selection of best suited cultivars, and optimal harvesting time (when content of active compounds is at its maximum) of proper plant parts.

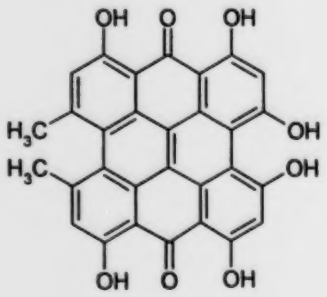
Analysis of St. John's Wort is a real challenge for an analyst, since this herb synthesizes a great number of physiologically active compounds, some of them at very low levels. An HPLC assay, developed in 1998 and improved in 1999, included seven markers: hypericin, a unique compound for this plant, and hyperoside, rutin, quercitrin, quercetin, chlorogenic acid and amentoflavone, which are more widely distributed compounds in plant kingdom. Their chemical structures are presented in Figure 21. Due to high difference in polarities of hypericin on one hand and other flavonoid compounds on the other, it was difficult to establish one set of conditions that would allow for baseline separation of all markers. A chromatographic profile for a standard mixture of these compounds is presented in Figures 22 and 23, and a typical profile of Saint John's Wort flower extract is presented in Figures 24 and 25.

In 1998 we initiated a study with an objective to establish influence of plant part and time of harvest on phytomedicinal quality of Saint John's Wort. Two and three years old plants, variety Standard, were grown in Herb Demonstration Garden, University of Saskatchewan, from seed purchased at Richters Herbs, and were harvested two times a day at seven different times from budding to post-blooming (June through September)(Table 4). Content of hypericins and 5 selected flavonoids in flowering tops (top 25 cm of the plant), upper leaves and stems (20 cm below), and lower leaves and stems (further 20 cm below), were used as quality parameters. The results are presented in Figures 26, 27, 28 and 29 and Table 5. The flowering tops followed by the upper most leaves contained the highest concentration of hypericins and flavonoids when harvested in late June, 0.48 % and 3.8%, respectively. The content of both groups of compounds was slightly higher in 2 years than in 3 years old plants. The hypericins content declined by more than 90% between late June and the end of August. The content of selected flavonoids, particularly rutin and hyperoside, showed a similar declining trend with increasing plant maturity for both 2 and 3 year old plants. The time of day (morning vs. afternoon harvest) did not seem to have a significant impact on phytomedicinal quality of plants. Second harvest of flowering tops in late August appeared possible, however, it caused higher susceptibility to winter kill and negatively impacted growth

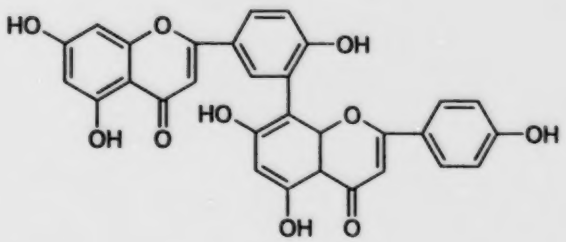
Figure 21. Chemical Structures of Marker Compounds in St. John's Wort



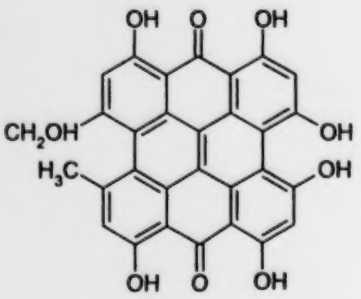
Quercetin R = H
Hyperoside R = Galactose
Quercitrin R = Rhamnose
Isoquercitrin R = Glucose
Rutin R = Rhamnose-Glucose



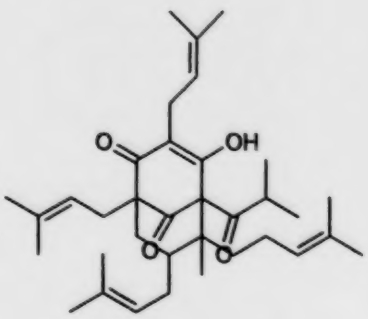
Hypericin



Amentoflavone



Pseudohypericin



Hyperforin

Figure 22. HPLC Profile of Hypericin Standard (absorbance at 592 nm)

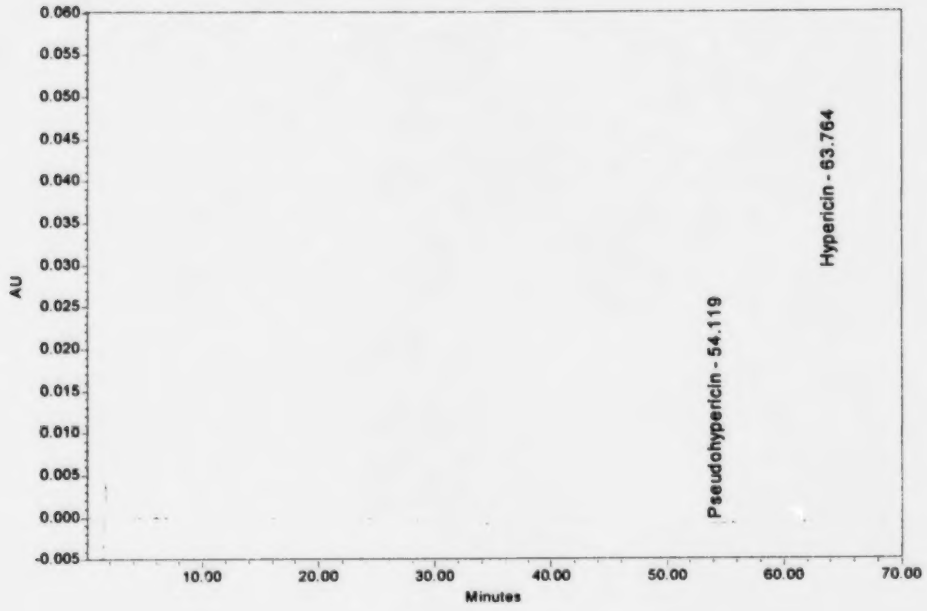


Figure 23. HPLC Profile of Flavonoid Standards (absorbance at 351 nm)

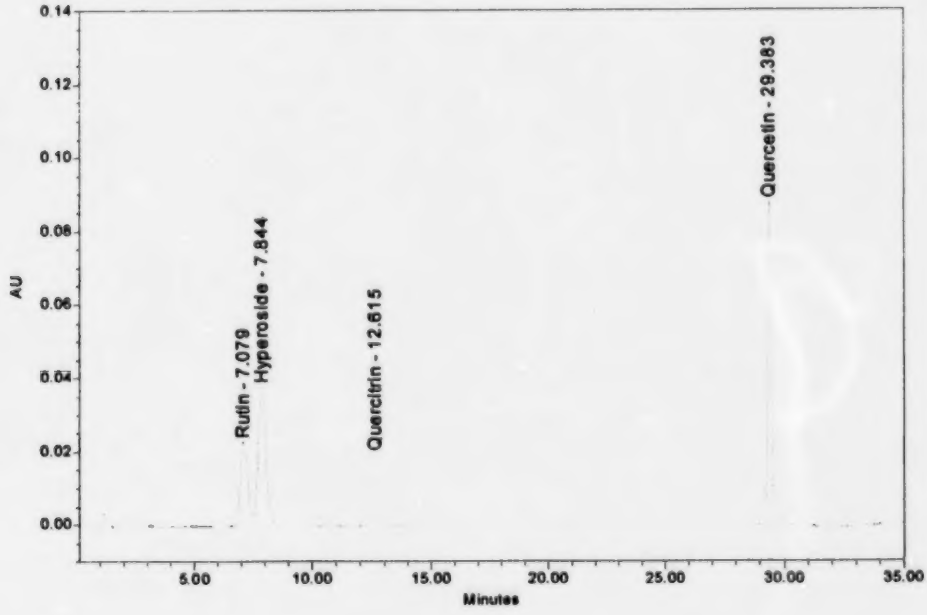


Figure 24. Typical HPLC Profile of St. John's Wort Flowers (absorbance at 592 nm)

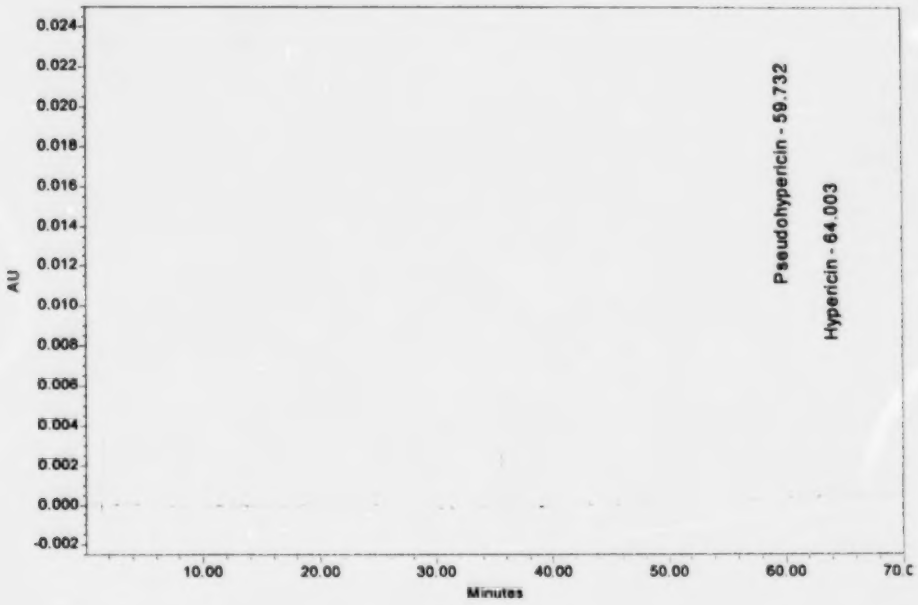


Figure 25. Typical HPLC Profile of St. John's Wort Flowers (absorbance at 351 nm)

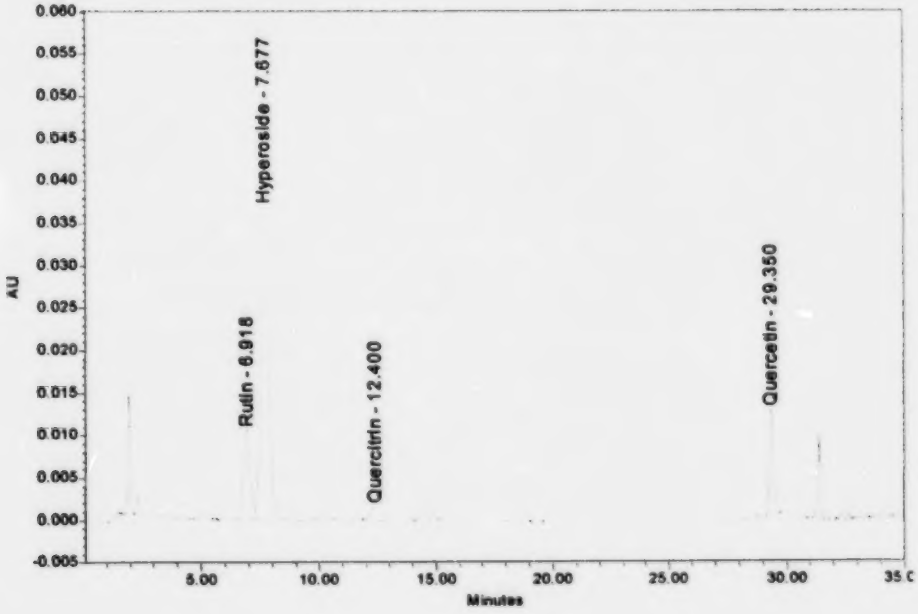


Table 4. Effect of Harvest Time on Quality of St. John's Wort[1,2] - Study Design

Parameter	Description
Plant age:	2 and 3 year old
Plant part:	Flowers, upper leaves and stems (top 10 cm), lower leaves and stems, flowering tops (upper 30 cm, parts not separated)
Date of harvest:	June 29, July 6, July15, July 23, July 30, Aug. 11, Aug. 20, Sept. 4, Oct. 7, Oct. 23
Time of day:	9 a.m., 4 p.m.
Date of second harvest:	Sept. 4, Oct. 7, Oct. 23 (row was cut back to 15 cm on July 30 and flowering tops reharvested)

[1] Content of hypericins and 5 selected flavonoids as indicators of phytomedicinal quality were determined in 84 samples of St. John's Wort

[2] Seed source: "standard" St. John's Wort, Richter's Herbs, ON

the following year. Plant quality was also compromised (Table 6).

In 1999 we investigated an effect of harvest time on quality characteristics of three St. John's wort cultivars that are available on the market: Topas (advertised as improved strain for commercial production, up to 0.5% hypericin, yields 2.5 tonnes/ha), Anthos (advertised as highly uniform strain, suited for mechanical harvest, disease resistant) and Elixir ™ (advertised as having higher active constituents, hypericin and related compounds, Richters exclusive). The plant material of these cultivars was kindly provided by Alberta Agriculture Crop Diversification Centre-South in Brooks, which also provided yield data (dry matter in kg/ha): about 1600 for Anthos, 900 for Topas and 2800 for Elixir ™. The results of testing for hypericins and flavonoid contents in our laboratory are presented in Tables 7 and 8. Considering both yield and quality, it appears that varieties Anthos and Elixir ™ should preferably be considered for cultivation. No comparative data of these three varieties and Standard variety are available at present.

Figure 26. Effect of Harvest Time on Hypericins Content in St. John's Wort Flowering Tops (2 and 3 year old plants)

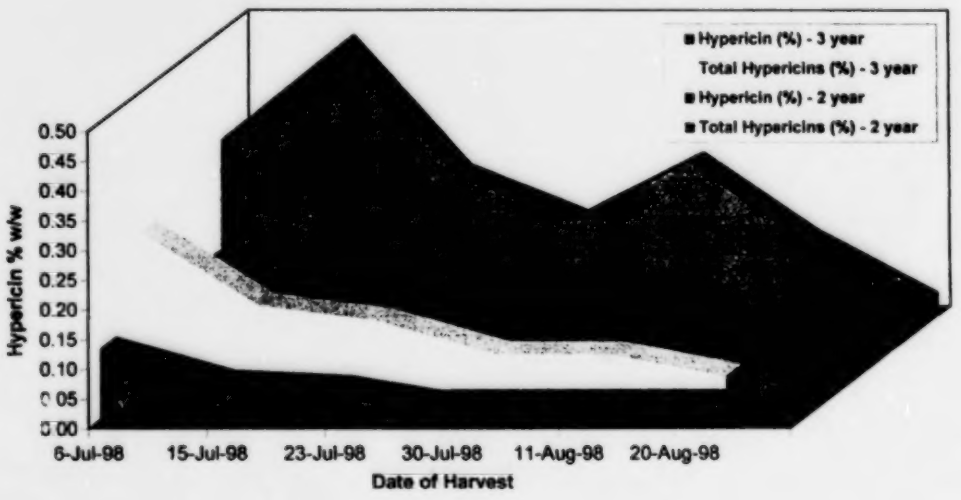


Figure 27. Effect of Harvest Time on Flavonoids Content in St. John's Wort Flowering Tops (2 year old plants)

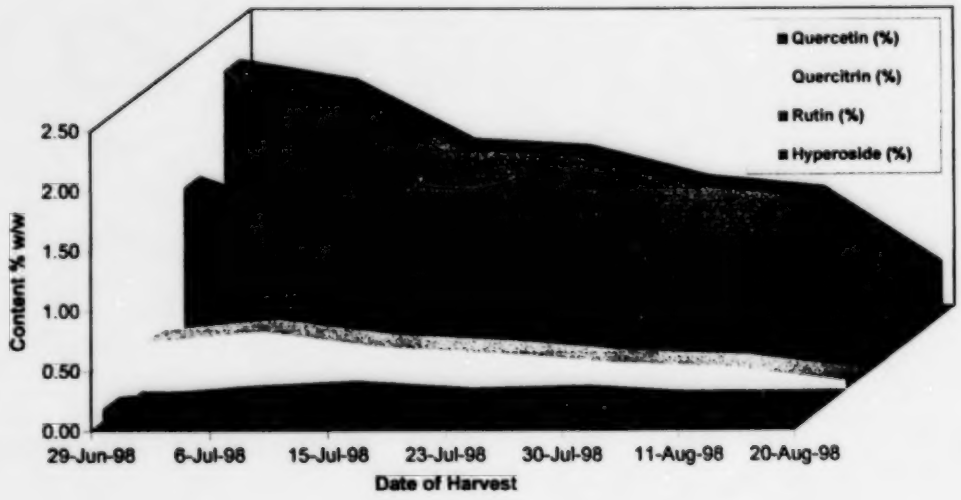


Figure 28. Effect of Harvest Time on Hypericins Content in Plant Parts of St. John's Wort (2 year old plants)

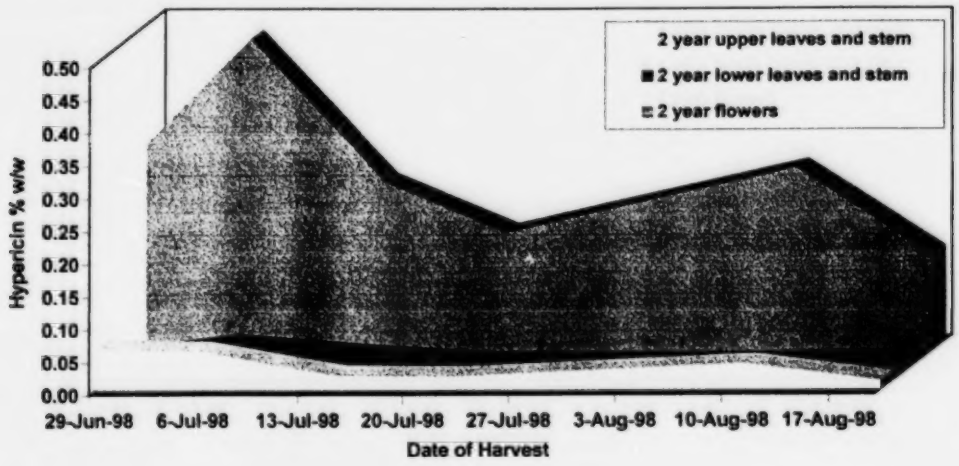


Figure 29. Effect of Harvest Time on Hypericins Content in Plant Parts of St. John's Wort (3 year old plants)

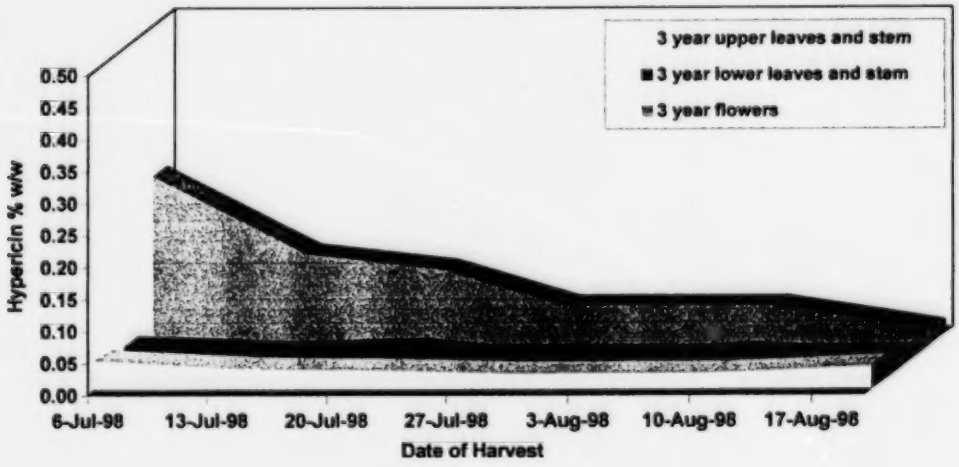


Table 5. Analysis of St. John's Wort Flowers (1998 Harvest)

Sample ID	Date of Harvest	Moisture (%)	Hypericin[1] (%)	Pseudohypericin[1,2] (%)	Total Hypericins[1,3] (%)	Quercitrin[1] (%)	Quercetin[1] (%)	Rutin[1] (%)	Hyperoside[1] (%)	Biapigenin[1] (%)
Flowers 2 yr.										
#5 HRP	29 Jun 98	11.21	0.139	0.179	0.318	0.407	0.106	1.439	2.161	0.026
#12 HRP	6 Jul 98	8.75	0.262	0.215	0.477	0.495	0.188	1.087	1.994	0.020
#20 HRP	15 Jul 98	8.91	0.133	0.125	0.258	0.366	0.237	0.842	1.499	0.015
#29 HRP	23 Jul 98	8.03	0.098	0.083	0.181	0.317	0.178	0.684	1.439	0.010
#48 HRP	30 Jul 98	12.80	0.132	0.147	0.279	0.235	0.198	0.453	1.188	0.011
#65 HRP	11 Aug 98	12.41	0.075	0.070	0.145	0.195	0.137	0.433	1.089	0.010
#81 HRP	20 Aug 98	10.91	0.021	0.019	0.040	0.075	0.143	0.210	0.457	0.012
Flowers 3 yr.										
#8 HRP	6 Jul 98	8.63	0.120	0.145	0.265	0.427	0.230	0.857	2.081	0.021
#16 HRP	15 Jul 98	8.26	0.064	0.078	0.142	0.313	0.234	0.656	1.613	0.014
#26 HRP	23 Jul 98	8.19	0.055	0.062	0.117	0.263	0.212	0.623	1.566	0.011
#45 HRP	30 Jul 98	12.75	0.023	0.038	0.061	0.096	0.156	0.387	0.927	0.006
#61 HRP	11 Aug 98	11.32	0.024	0.036	0.060	0.078	0.135	0.329	0.786	0.007
#77 HRP	20 Aug 98	11.62	0.009	0.012	0.021	0.044	0.096	0.228	0.621	0.007

[1] Content of hypericin, pseudohypericin, total hypericins, quercitrin, quercetin, rutin, hyperoside and biapigenin are reported on dry weight basis.
[2] Pseudohypericin was quantitated using the calibration curve for hypericin, assuming a similar ε value.
[3] Total hypericins were expressed as the sum of hypericin and pseudohypericin.

Table 6. Quality of Second Harvest of St. John's Wort Flowers

Sample ID	Date of 2nd Harvest	Moisture (%)	Hypericin[1] (%)	Pseudohypericin[1,2] (%)	Total Hypericins[1,3] (%)	Quercitrin[1] (%)	Quercetin[1] (%)	Rutin[1] (%)	Hyperoside[1] (%)	Biapigenin[1] (%)
Flowers 2 yr.										
#109 HRP	4 Sep 98	6.77	0.124	0.119	0.243	0.263	0.212	0.623	1.566	0.011
#112 HRP	4 Sep 98	8.38	0.034	0.067	0.101	0.114	0.084	0.895	0.775	0.008
Leaves and Stem 2 yr.										
#110 HRP	4 Sep 98	6.11	0.034	0.034	0.068	0.473	0.046	0.757	0.997	Trace
#113 HRP	4 Sep 98	7.03	0.019	0.030	0.049	0.252	0.042	1.526	0.887	Trace

[1] Content of hypericin, pseudohypericin, total hypericins, quercitrin, quercetin, rutin, hyperoside and biapigenin are reported on dry weight basis.
[2] Pseudohypericin was quantitated using the calibration curve for hypericin, assuming a similar ε value.
[3] Total hypericins were expressed as the sum of hypericin and pseudohypericin.

Table 7. Effect of Harvest Time and Variety on Hypericins Content in St. John's Wort (*Hypericum perforatum)*

Variety	Date of Harvest	Hypericin (%)	Pseudohypericin (%)	Total Hypericins (%)
2 year				
Anthos	6-Jul-99	0.026	0.071	0.097
Topas	6-Jul-99	0.015	0.040	0.055
Elixir™	6-Jul-99	0.025	0.049	0.074
Anthos	17-Sep-99	0.014	0.030	0.044
Topas	17-Sep-99	0.009	0.018	0.026
Elixir™	17-Sep-99	0.019	0.029	0.048

Table 8. Effect of Harvest Time and Variety on Flavonoids Content in St. John's Wort (*Hypericum perforatum)*

Variety	Date of Harvest	Quercitrin (%)	Quercetin (%)	Rutin (%)	Hyperoside (%)
2 year					
Anthos	6-Jul-99	0.102	0.025	0.737	0.913
Topas	6-Jul-99	0.078	0.018	0.921	0.775
Elixir™	6-Jul-99	0.097	0.031	0.662	1.086
Anthos	17-Sep-99	0.043	0.021	0.738	0.641
Topas	17-Sep-99	0.042	0.011	0.832	0.601
Elixir™	17-Sep-99	0.082	0.067	0.719	0.909

4. Calamus Root - Toxicity Assessment of Saskatchewan Wild Populations

Calamus root/rhizome (*Acorus calamus*) is widely used as a herbal remedy by First Nations people. Because certain chemotypes of calamus contain a carcinogenic compound known as β-asarone, it was of interest to assess whether local Saskatchewan populations present a health hazard due to its toxicity. In our earlier studies on small number of samples of calamus root we found that one population from the Hudson Bay area contained considerable amounts of β-asarone, while one from La Ronge contained no β-asarone, but only α-asarone, a non-toxic asarone isomer. This finding suggested that content of β-asarone varies and that it would be worthwhile pursuing a more comprehensive study on this subject to identify chemotypes with no toxicity.

Samples of calamus root were collected at seven sites across Saskatchewan as presented in Table 9. They were ground and subjected to essential oil determination. The recovered essential oil

was analyzed by gas chromatography-mass spectrometry (GC-MS) in the past and MS spectra generated then were used to positively identify peaks of asarone isomers in the current study.

Table 9. Sources of *Acorus calamus* Rhizomes

Location	Year of Collection	Time of Collection
La Ronge, SK	1999	June 23
La Ronge, SK	1998	September 16
La Ronge, SK	1997	July 01
Ilex, SK	1998	September 22
Denare Beach, SK	1997	July 15
Besnard K60, SK	1997	July 02
Mistik Lake, Creighton, SK	1997	May 14

Determination of essential oils Dry roots were cut in small pieces, immersed into liquid nitrogen (N_2) and ground into fine powder with a coffee grinder. The fine powder was passed through a No. 10 (2 mm, 9 mesh) sieve and placed in a 2000 mL round bottom flask to which distilled deionized water was added. The ratio of water to powder was 10:1. Essential oil was distilled using co-habitual hydrodistillation of a Clavenger-type over 4.5 hrs. To eliminate any loss of highly volatile compounds during the lengthy distillation, a dry ice condenser-trap was added to the top of the condenser. The essential oils from all samples were extracted from the distillate with diethyl ether since the oil was of higher density than water and had settled to the bottom of the receiver.

Gas Chromatography (GC) analysis GC analysis was conducted on a Hewlett Packard 5890 Series II gas chromatograph equipped with a flame ionization detector. The column used was a 30 m x 0.32 mm i.d., 0.25 mm film thickness HP-5 [crosslinked (5%)-diphenyl-(95%)-dimethylsiloxane copolymer] fused-silica capillary column. The carrier gas was helium and temperature programming was as follows: initial temperature 75°C for 3 min, elevated to 250°C at a rate of 5°C/min, kept for 2 min at 250°C then raised to 300°C at a rate of 20°C/min, kept for 5 min. The inlet and detector temperatures were 250°C. The split ratio was 35:1. Prior to analysis essential oils were dissolved in hexane and 1 mL was injected into the column. The asarone standard (70% β-asarone and 30% α-asarone) was used to generate calibration curve in the range of 0.75 μg/mL to 160.5 μg/mL. Millennium[32] Chromatography software was used for processing of chromatography data.

The yield of essential oil and content of two asarone isomers are presented in Table 10. The GC profile of essential oil from *Acorus calamus* rhizomes (La Ronge 1999), which is typical for Saskatchewan grown calamus is presented in Figure 30 (retention time of β-asarone is 22.05 min

and α-asarone 23.07 min). These data suggest that content of β-asarone is not high enough to cause a concern based on our current knowledge of β-asarone toxicity.

Table 10. Yield and Composition of Essential Oil from *Acorus calamus* Rhizomes

Source	Yield of Essential Oil (% w/w)[1]	Amount of α-asarone (%)	Amount of β-asarone (%)
La Ronge 1999	2.89	0.243	0.124
La Ronge 1998	1.33	Trace	0.112
La Ronge 1997	2.55	0.315	0.199
Ilex 1998	1.27	0.242	0.136
Denare Beach 1997	1.23	trace	0.137
Besnard K60 1997	2.80	trace	0.139
Mistik Lake 1997	2.73	trace	0.126

[1]on wet weight basis

5. Calendula

Calendula (*Calendula officinalis*) is a well established medicinal plant species with well documented anti-inflammatory and other biological activities. The local company uses calendula extract (typically imported from Europe) in its unique "Foot Fresh" preparation promoted to control sweating of feet. Also, calendula is presently being evaluated as an oilseed crop by CDC, since it contains an unusual fatty acid known as calendic acid. This fatty acid is the most rapidly oxidized fatty acid known in nature with variety of potential applications in the plastics, paint and coating industries. Consequently, it is of no surprise that study on large scale cultivation of calendula with potential dual application, medicinal and industrial, has been initiated in 1999 in Saskatchewan.

Our interest in calendula is focused on accumulation of flavonol-glucosides and essential oil as active compounds and quality parameters. We initiated development of an HPLC method for quantitation of isorhamnetin-3-glucoside, isorhamnetin-3-rutinoside, and rutin, believed to be "active"/marker compounds for calendula. An effect of plant maturity, i.e., stage of flower development, on flavonol glycoside was also studied. The results presented in Table 11 and 12 suggested that for medicinal markets calendula flowers should be harvested soon after the buds start opening and when concentration of flavonol-glucosides reached its maximum, and not when flowers

Figure 30. GC Profile of Essential Oil from *Acorus calamus* Rhizomes (La Ronge 1999)

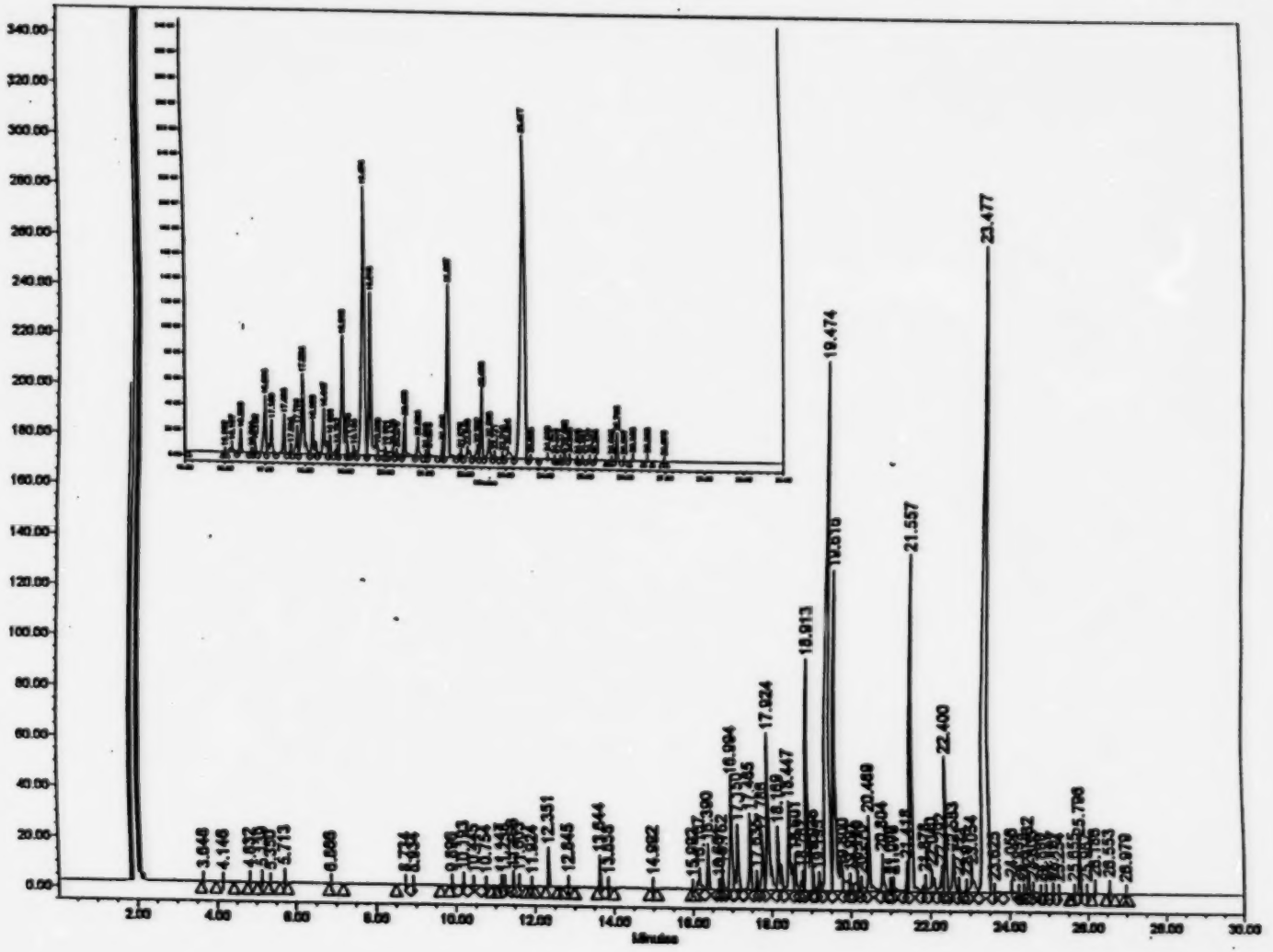


Table 11. Effect of Stage of Maturity on Flavonol Glycoside Content in *Calendula officinalis* Grown in Saskatoon, Canada

Stage of Maturity	Buds Closed	Buds Open	Full Bloom	Past Full Bloom	Seed Heads
I-3-rutinoside[1] (%)	0.382	0.498	0.365	0.157	0.013
I-3-glucoside[1] (%)	0.092	0.048	0.035	0.019	ND

[1] Contents of I-3-rutinoside and I-3-glucoside are reported on a dry weight basis.
ND - not detectable.

Table 12. Effect of Stage of Maturity on the Composition of Essential Oil from *Calendula officinalis* Grown in Saskatoon, Canada

Stage of Maturity	Buds Closed	Buds Open	Full Bloom	Past Full Bloom	Seed Heads
Essential Oil[1] (%)	0.25	0.27	0.34	0.35	0.07
Cadinol[2,4] (%)	49.02	47.10	52.14	51.37	47.95
Cadinene[2,3] (%)	11.97	14.43	11.34	12.19	9.86
Ledol[2] (%)	1.12	0.85	1.08	1.03	1.29
Hexafarnesyl Acetone[2] (%)	0.81	1.45	0.70	0.63	0.19
Copaene[2] (%)	0.29	0.32	0.27	0.29	0.08
Caryophyllene[2] (%)	0.29	0.62	0.36	0.31	0.11

[1] Content of essential oil is reported on per dry weight basis.
[2] Contents of essential oil components are reported on a relative basis.
[3] Cadinene is comprised of four isomers.
[4] Cadinol is comprised of two isomers.

are in full bloom, as commonly believed. Plants are of no medicinal value once the seed start forming. A study aimed at identifying other flavonol-glucosides present in calendula flower extract, beside isorhamnetin-3-glucoside and isorhamnetin-3-rutinoside (the only two standard compounds available commercially) using LC-MS on a fee for service basis has been pursued. Results are expected later in the year 2000.

6. Burdock, Licorice and Astragalus – Quality Assessment

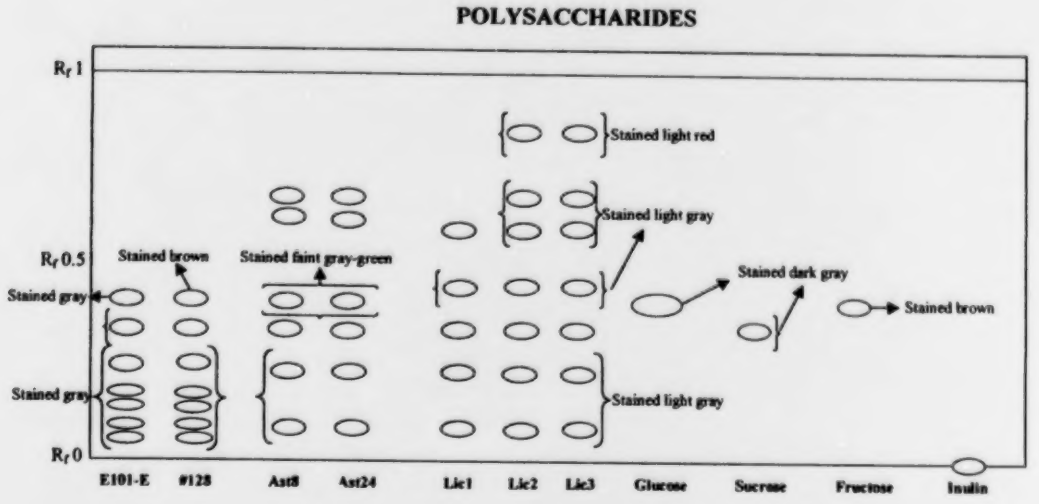
Burdock, licorice and astragalus are also considered to be promising medicinal root crops for Saskatchewan. It has been established with certainty that all three species can be easily grown locally, but the quality of the material needs to be determined. Licorice is a perennial plant and various species have been used medicinally: *Glycyrrhiza glabra* (common), *Glycyrrhiza uralensis* (Chinese), *Glycyrrhiza lepidota* (Saskatchewan wild). All three species have been grown successfully at our Herb Research and Demonstration Garden over the last three years. Some herb growers in Saskatchewan have been growing experimentally common licorice too. *Glycyrrhiza lepidota* is widely distributed throughout Saskatchewan and it was of interest to establish extent of differences in chemical composition between the licorice roots used in herb trade (common and Chinese), and wild Saskatchewan species. Licorice root has been used worldwide for ulcer and stomach distress, and inflammatory problems such as arthritis. It is one of the most prescribed drugs in Chinese medicine. Burdock is a biannual plant whose root can be harvested in year 1 and 2 for medicinal purposes. Wild burdock (*Arctium minus*) is widely distributed in Saskatchewan, but is considerably smaller from its cultivated relative. Burdock is used for a variety of medical conditions, but is best known for its body cleansing properties, as a diuretic and laxative. Astragalus, also known as a Chinese milkvetch, is a perennial herb whose root is typically harvested in its third year. It is very commonly grown in China and its primarily used for its immunostimulatory properties. It is used in numerous Chinese prescriptions.

Licorice [*Glycyrrhiza glabra* (common), *Glycyrrhiza uralensis* (Chinese), *Glycyrrhiza lepidota* (Saskatchewan wild)]

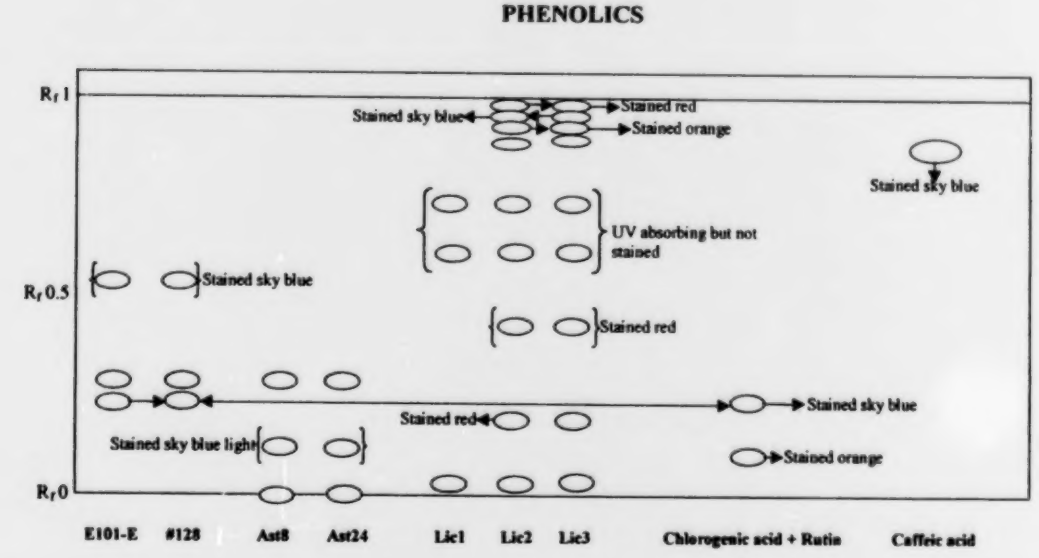
According to the literature main active compound in licorice root is glycyrrhizin with saponin-like properties, also known as glycyrrhizic acid (commonly present at 4-7 % in the root, but can reach up to 14%). Root also contains flavonoids particularly flavonol glycosides, polysaccharides and some other minor compounds.

The TLC profiles of methanolic root extracts of all three licorice species (Figures 31 and 32) suggested that all three species contain saponins, phenolics (flavonoids included) and polysaccharides, as expected. A high resemblance in TLC profiles between common and Chinese licorice for all three classes of natural compounds (indicating similar chemical composition) suggest that these two species of licorice could likely be used interchangeably for various medical conditions. Unfortunately, wild Saskatchewan licorice appears to contain considerably lower content

Figure 31. TLC Profiles of Polysaccharides (a), Phenolics (b) from Roots of *Arctium lappa*, *Astragalus membranaceous* and *Glycyrrhiza* spp.

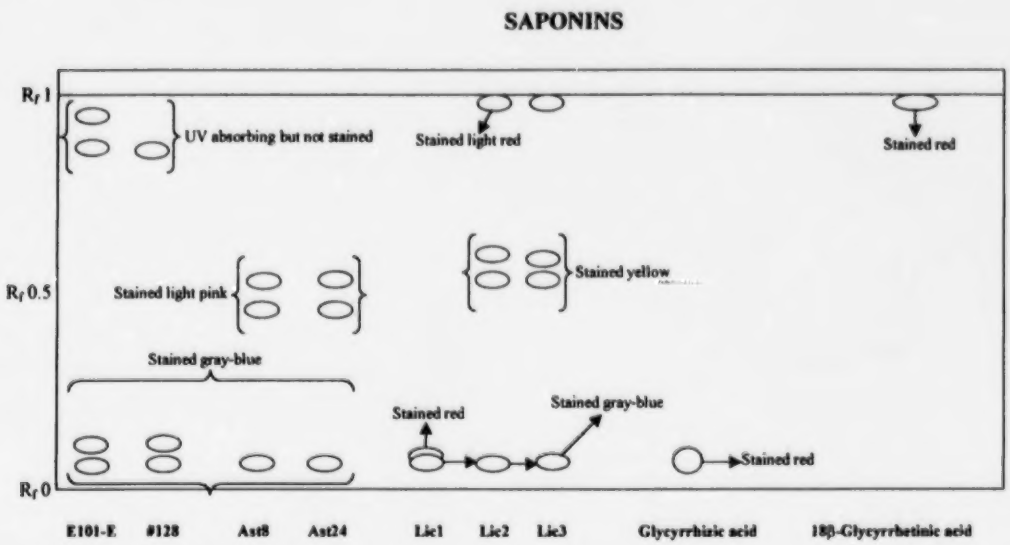


Labels: E101-E: Burdock root (NorSask Botanicals); #128: Burdock root (Herb Research Garden); Ast8: *A. membranaceous* root (8 hrs extraction); Ast24: *A. membranaceous* root (24 hrs extraction); Lic1: *G. lepidota* root; Lic2: *G. glabra* root; Lic3*: G. uralensis* root



Labels: E101-E: Burdock root (NorSask Botanicals); #128: Burdock root (Herb Research Garden); Ast8: *A. membranaceous* root (8 hrs extraction); Ast24: *A. membranaceous* root (24 hrs extraction); Lic1: *G. lepidota* root; Lic2: *G. glabra* root; Lic3*: G. uralensis* root

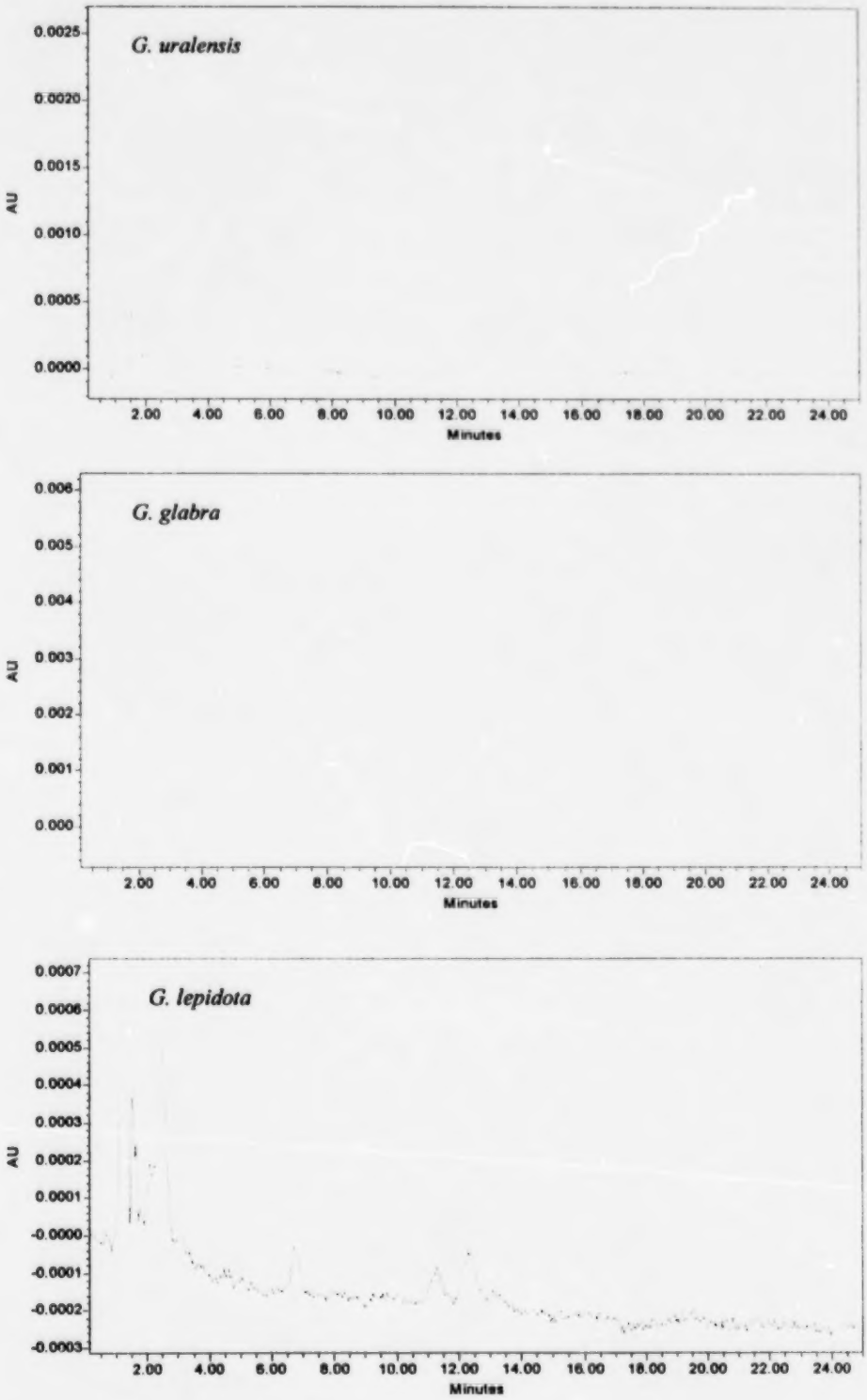
Figure 32. TLC Profiles of Saponins from Roots of *Arctium lappa*, *Astragalus membranaceous* and *Glycyrrhiza* spp.



Labels: E101-E: Burdock root (NorSask Botanicals); #128: Burdock root (Herb Research Garden); Ast8: *A. membranaceous* root (8 hrs extraction); Ast24: *A. membranaceous* root (24 hrs extraction); Lic1: *G. lepidota* root; Lic2: *G. glabra* root; Lic3*: G. uralensis* root

of phenolics and saponins (but appears to contain medicinally important glycyrrhizin) and somewhat lower content of polysaccharides. Wild Saskatchewan licorice also appear to have considerably different flavonoid profile than both common and Chinese licorice (Figure 33). The resemblance in flavonoid profile of the latter two species further suggest that common and Chinese licorice are more closely related in their chemical composition. The profiling of natural compounds in three species of licorice to date does not support possible replacement of widely used common licorice with its wild Saskatchewan counterpart. Determination of glycyrrhizin content in all three species of licorice by HPLC method will be pursued in the near future, and is expected to shed some light on the possible applications for local licorice.

Figure 33. HPLC Profiles of *Glycyrrhiza* spp. (absorbance at 351 nm)



Astragalus (*Astragalus membranaceous*)

According to the literature, polysaccharides are considered to be primarily responsible for the immunostimulatory effects of astragalus. We tested a methanolic root extracts tested for the presence of polysaccharides, saponins and phenolics using TLC. The TLC chromatograms indicated presence of all three classes of compounds with polysaccharides being the dominant ones (Figures 31 and 32). Future studies will concentrate on closer examination of all three classes of compounds.

Burdock (Great burdock *Arctium lappa or A. majus;* Lesser burdock *A. minus)*

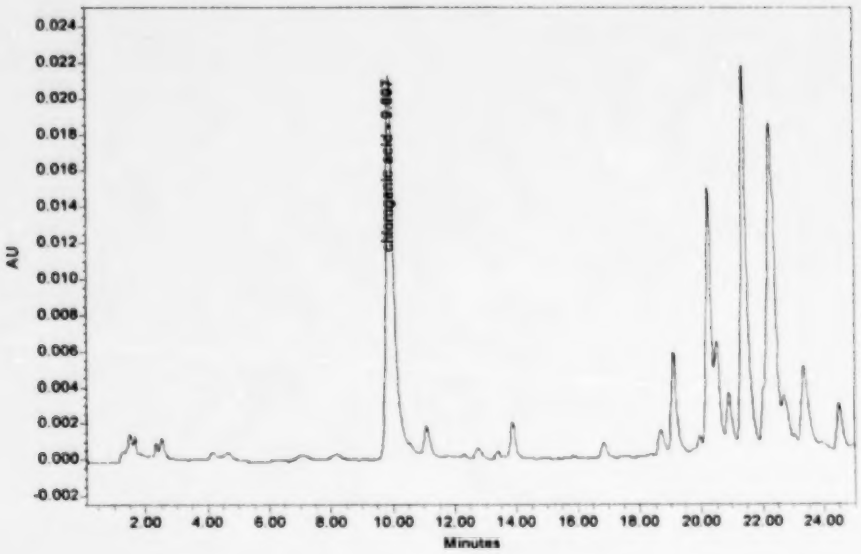
According to the literature, burdock contains 27-45% inulin (polysaccharide), 2-4% of polyphenolic compounds, polyacetylenes, some essential oil, and other minor compounds. The TLC profiles of methanolic root extracts tested for the presence of polysaccharides, phenolics and saponins supported presence of first two classes of natural compounds, but not saponins (Figures 31 and 32). The HPLC testing for the presence of polyphenolic compounds revealed that chlorogenic acid is one of four major phenolic acids in burdock (Figure 34). The content of chlorogenic acid in two samples of burdock root cultivated in Saskatchewan and 3 samples from Davis, California, were in the range 0.23-0.34%; age didn't seem to influence the chlorogenic acid content (Table 13).

Table 13. Chlorogenic Acid Content of Burdock Root

Source	Moisture Content (% w/w)	Weight of Dry Extract[1] (g)	Chlorogenic acid[2] (% w/w)
Norsask Botanicals - (1 year)	7.27	0.25	0.34
Herb Research Garden (2 year)	8.03	0.19	0.23
#1 Davis, CA - (1 year)	9.19	0.22	0.26
#2 Davis, CA - (1 year)	11.07	0.26	0.23
#3 Davis, CA - (1 year)	11.66	0.29	0.34

[1] weight expressed in g of dry extract per g of root as provided (as is)
[2] on dry weight basis

Figure 34. HPLC Profile of Chlorogenic Acid in Burdock Root

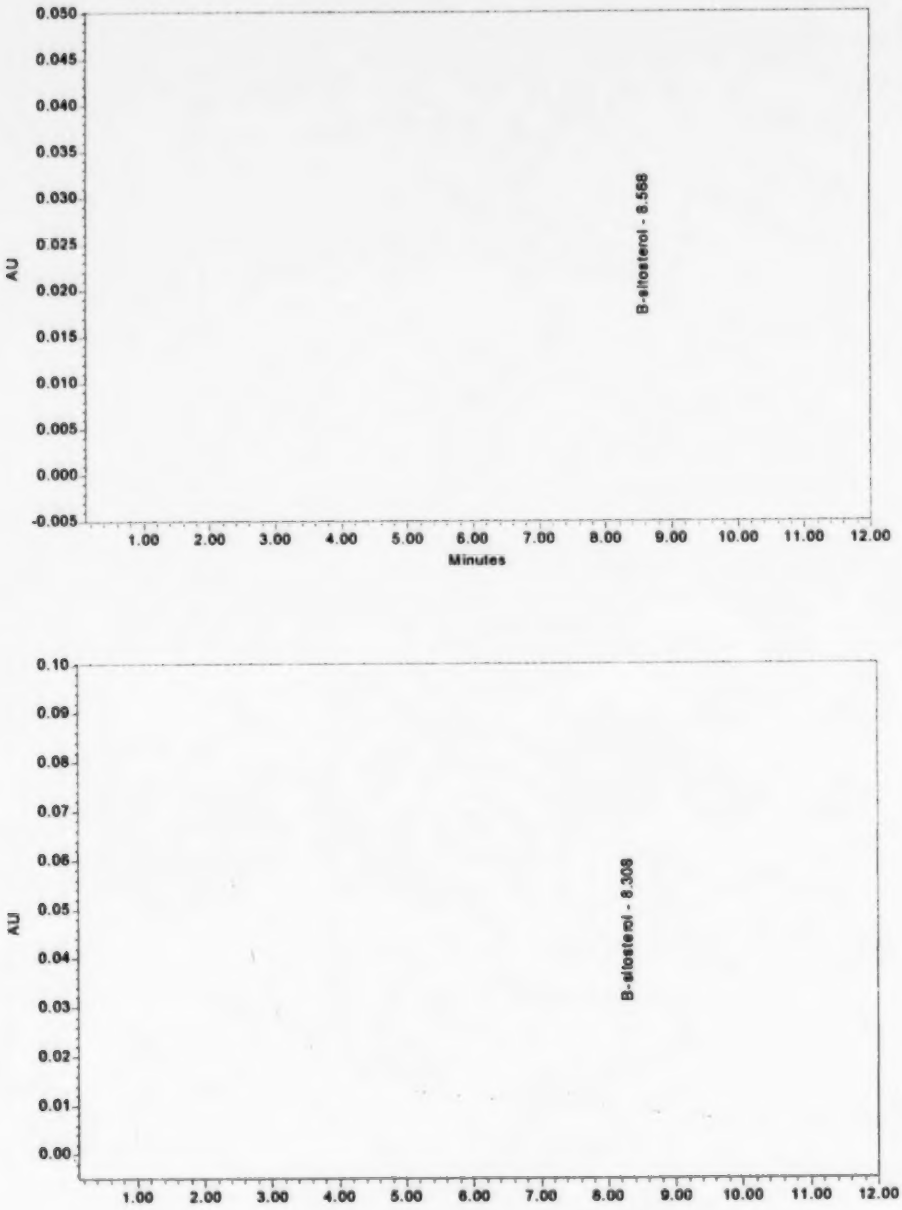


7. Stinging Nettle – Quality Assessment

Root extracts of stinging nettle (*Urtica dioica*) are used in the treatment of benign prostatic hyperplasia (BPH). There is some evidence that the presence of lignans is responsible for the physiological effect of this herbal drug. Also phytosterols, such as ß-sitosterol, have been proven to be effective drugs for the treatment of stage I to II BPH symptoms.

With this background, an effort was made to develop an HPLC assay for quantification of ß-sitosterol in stinging nettle root. The representative HPLC profile of methanolic root extract is shown in Figure 35. In preliminary studies root was found to contain 0.167% (dry weight basis) of ß-sitosterol. In future studies larger sample pool will be included and other constituents beside ß-sitosterol will be considered for analysis. Stinging nettle is highly promising medicinal plant for the province.

Figure 35. HPLC Chromatogram of β-Sitosterol Standard (upper panel) and Stinging Nettle Root Extract (lower panel), detection at λ = 210 nm.



8. Feverfew – Quality Assessment

We developed an HPLC method for analysis of parthenolide in 1995 and have used it for various research projects, past and ongoing, as well as an analytical service to the industry. Flowers of feverfew (*Tanacetum parthenium*) were found to contain the highest level of parthenolide, up to 1.41% at full bloom. Leaves contained up to 0.25% and stems up to 0.02% parthenolide at preflowering stage. Flowering tops consisting of flowers and leaves (upper 1/3 to 1/2 of the plant), the most common form in trade, contain up to 0.96% of parthenolide. It is a common occurrence that content of parthenolide varies considerably from one sample to another and little has been offered in the literature to assist with understanding of the causes. In general, high quality feverfew can be grown in Saskatchewan as a viable annual medicinal crop.

9. Milk Thistle - Quality Assessment

In 1995, the SHRP developed an HPLC method for analysis of milk thistle (*Silybum marianum*) seed for the content of silymarin, a flavonolignan compound considered to be active ingredient; the assay has been used since for various research projects, past and ongoing, as well as an analytical service to the industry. Milk thistle seed contains silymarin consisting of silibinin, silychristine, silydianine and taxifolin in the range of 1.02-2.73% and fixed oil in the range of 17.4-26.2%. In general, good quality milk thistle can be grown in Saskatchewan as a viable annual medicinal crop.

10. German Chamomile - Quality Assessment

German chamomile (*Matricaria recutita*) blossom has been analyzed for the content and composition of blue coloured essential oil by gas chromatography. It typically yields 0.4-0.8% oil, which consists of camazulene (8.17-20.74%), bisabolone oxide, beta-farnesene and 2-furan methanol as main constituents. It is believed that German chamomile selections from Europe yielding higher amount of essential oil with favourable composition should be accessed and tested in Saskatchewan. Determination of flavonoid composition in German flowers has been attempted, but further research is required.

11. Valerian - Quality Assessment

In 1995, the SHRP developed a chromatographic assay for marker compounds of *Valeriana officinalis* root, valerenic and acetoxy-valerenic acids. We have used it since for various research projects, past and ongoing, as well as an analytical service to the industry.

Saskatchewan climate is well suitable for commercial cultivation of valerian. The samples collected from various locations in Saskatchewan showed a mean valerenic acid content of 0.2% on a dry weight basis, but content can be as high as 2.5%. Acetoxy-valerenic acid could not be quantified as no reference compound is commercially available, however, it is present in an approximately equal concentration as valerenic acid. The valerenic acid content compares favorably with the 0.08-0.3% of total valerenic and acetoxyvalerenic acid reported elsewhere. The content of essential oil in the root ranges from 0.5% to 0.9%, which also compares favorably with the expected yield of 0.3-0.7%. In the last 2-3 years diseases such as aster yellows and a root rot, caused by an unidentified pathogen, had a tremendous impact on valerian root crop. In general, high quality valerian can be grown in Saskatchewan as a viable perennial medicinal root crop. However, disease management requires an immediate attention before commercial cultivation takes off.

12. American Ginseng - Quality Assessment

An HPLC method has been developed by SHRP in 1996/97 for analysis of 5 ginsenosides, considered to be active constituents in North American ginseng (*Panax quinquefolius*). The root grown in Saskatchewan and Manitoba was found to contain up to 3.26% ginsenosides, which is considered an average quality. Ginseng is, however,

13. Analytical Services to Herb Industry

The summary of all services provided since May 1997 is presented in Table 14.

Table 14. Summary of Analytical Services Provided May 1997 – January 2000

Medicinal Plant Sample	Service Requested	May 97-July 97	Aug 97–Jan 98	Feb 98–July 98	Aug 98–Jan 99	Feb 99–July 99	Aug 99–Jan 00	TOTAL
Echinacea spp. Root	Species Identification	6	9	3	22	4	3	47
E. angustifolia Root	Quality Analysis	0	2	11	14	14	19	60
E. pallida Root	"	0	0	3	3	0	0	6
E. purpurea Root	"	0	2	0	0	2	2	6
E. angustifolia Aerial Parts	"	0	0	1	0	1	0	2
E. angustifolia Extracts	"	0	1	16	2	12	9	40
E. angustifolia Extracts	Total Phenolic Content	0	0	0	1	1	0	2
E. angustifolia Commercial Products	Quality Analysis	0	0	0	0	2	1	3
E. purpurea Aerial Parts	"	0	2	0	0	2	2	6
E. purpurea Extract	"	0	2	3	2	7	2	16
Echinacea spp. Complex Extract	"	0	0	0	0	0	2	2
Echinacea spp. Plant Material (unspecified)	"	0	0	0	0	0	1	1
Echinacea spp. Seed	Species Identification	0	0	7	13	25	12	57
St. John's Wort Aerial Parts	Quality Analysis	0	0	2	0	3	7	12
St. John's Wort Extracts	"	0	0	1	0	5	3	9
St. John's Wort Capsules	"	0	0	0	0	1	0	1
Valerian Root	"	0	0	0	1	0	2	3
Feverfew Aerial Parts	"	0	0	0	0	2	0	2
Feverfew Commercial Products	"	0	0	0	0	0	12	12
Burdock Root	"	0	0	0	0	1	0	1
Milk Thistle Seed	"	0	0	0	0	1	0	1
Cilantro Essential Oil	"	0	2	0	6	0	16	24
Dill Essential Oil	"	2	7	0	2	0	0	11
Caraway Essential Oil	"	3	0	0	1	0	0	4
TOTAL		**11**	**27**	**47**	**67**	**83**	**93**	**328**

Since the introduction of the analytical services in summer 1997, the total of 328 samples were analyzed, 75% of which were echinacea related. Majority of tests were root quality assessment, followed by species verification of seed, species verification of root, and analysis of echinacea herbal extracts. The remaining analyses included analysis of essential oil from cilantro, dill weed, and caraway, analysis of feverfew plant and commercial preparations, St. John's Wort plant and final preparations, milk thistle seed, valerian root, burdock root etc. Lack of supply of *E. angustifolia* seed of guaranteed authenticity on the market caused a marked increase in demand for echinacea seed verification. The percentage of misidentified seed decreased considerably in 1999 in comparison to 1998 when seed testing was introduced. This is an encouraging new development. It is believed that an accessible and affordable echinacea testing provided by the Herb Research Program has contributed to the growing supply of *E. angustifolia* seed of reliable authenticity, and in turn to the credibility and competitiveness of Saskatchewan echinacea industry.

OBJECTIVE 2: to continue updating the Saskatchewan Herb Database, i.e. revising existing cultural, processing and marketing information and adding new herbs with economic potential.

Achievements

14. Extension and Other Information Dissemination

In early 1998 information has been drafted for comfrey, St. John's Wort, calendula, hawthorn and eleuthero (Siberian ginseng) as new entries for the 2nd expanded edition of Saskatchewan Herb Database. However, given a broader research mandate and involvement in provision of analytical services to the industry, it soon became apparent that SHRP lacked resources dedicated to the time consuming task of developing accurate written information. With the continuous expansion of the medicinal plant industry in Saskatchewan, there was an ever growing demand for information on herbs by interested growers and processors, and in particular, an increased demand for provision of analytical services by the Herb Research Laboratory, as elaborated under Objective 1 of this report.

It appears impossible for the SHRP to meet all of the set objectives by relying on the same personnel. Later in 1998, the Saskatchewan Herb and Spice Association received funding to prepare 2nd and expanded edition of the Herb and Spice Growers

Manual, first published in 1994. The target date was June 1999. This relieved pressure from the SHRP to prepare 2nd edition of Saskatchewan Herb Database. The need was no longer immanent as envisioned at the time this project was concieved in 1997. By the time this project gets completed (February 2000), industry should have an access to an up-to-date production and other information on at least 30 herb species that have good potential to be cultivated in Saskatchewan.

Two information sheets on Valerian and Milk Thistle were developed in fall 1997, and technical and editorial assistance was provided to Saskatchewan Agriculture and Food (Bryan Porter and Ray McVicar) to develop two new herb infosheets, on St. John's Wort and Feverfew in 1999. SHRP updates annually for the industry the following information:

-Medicinal Plant Seed/Seedling Supplier List Update, p.3
-List of Seed and Plug Suppliers of Echinacea, p.2
-Medicinal Plants Buyers and Marketers List Update, p.3
-List of Web Sites Relevant for Herb Industry, p.3
-Miscellanious informations on request.

The major extension services SHRP have provided to herb industry over the past 30 months included: over 1000 calls, 190 information mail-outs and over 100 electronic mail-outs, 128 office visits; over 30 organized group tours of Herb Resource and Demonstration Garden and over 30 unguided visits; 27 sample diagnostics; 12 field trips. The range of extension services was broad and included various aspects of production (e.g., seed sourcing, propagation, agronomic practise, harvesting, post-harvest handling), processing (e.g., forms of processing, equipment requirements, processing protocols, industry contacts), marketing (how to market and where, buyers contacts), and regulations (regulations requirements for various categories of herb products, labeling and label design). Great number of inquiries were quality related (e.g., what it means, how to maintain it, industry standards, how to prepare sample for testing). The SHRP continued to provide leadership in research and development activities through public speaking,

distribution of pertinent information developed internationally, guest lecturing, and consulting to various growers/interest groups.

Assistance in the form of consultation and provision of plant material was provided to Conservation Learning Centre in Prince Albert and Norsask Botanicals, Inc. in Tisdale. Consultation to many groups and companies such as Northern Lights Herbs, Inc. in Prince Albert, Wise Owl Herbs Co. Inc. in Unity, Coulee Pickings Inc. in Regina, Maple Ridge Farms Inc. in Prince Albert, MidNorthern Growers Ltd. in Moose Jaw, were also provided on diverse issues from seed authentication and production of medicinal plants to regulatory and labeling requirements.

15. Herb Demonstration and Resource Garden

Of great benefit to learning and writing about medicinal and aromatic plants is access to the Herb Demonstration and Resource Garden. It serves as a foundation on which to build a knowledge about medicinal plants suitable for Saskatchewan and beyond, and provides a great opportunity to test suitability of species as potential cash crops for Saskatchewan. The garden has been maintained in the University field headquarters since 1995. Summer student assistant was typically hired part time during summer months to assist in maintaining existing perennial beds, add several new species of annual and perennial herbs, and to survey the garden biweekly. Every effort was made to keep the garden presentable to visitors, who could visit the garden and view over 70 species of herbs throughout the summer.

OBJECTIVE 3: To continue research on Saskatchewan indigenous plants as source of bioactive compounds.

Achievements

16. Collaborative Projects

The activities with the above objective expanded over the time to include studies on discovering new phytomedicinals and nutraceuticals from broader range of plants and

for several biological activities. Two new collaborative projects were established, one with the Department of Applied Microbiology and Food Scvience and one with the Department of Pharmacology, College of Medicine.

16 a) Studies on Immunostimulatory Activities

The investigation of senega root as a potential source of commercially important immunostimulating compounds, specifically animal vaccine adjuvant (agent that improves vaccine efficacy) was continued. Testing of adjuvanticity of several senega saponin fractions with different degree of purity *in vivo*, dose dependency studies and histological studies on the muscle tissue surrounding the injection site were conducted. These studies are expected to reveal whether senega saponins could cause any possible toxic side effects, which would be of concern in potential animal and human applications. Also, some attention was given to probing formation of immune-stimulating complexes (ISCOMs), a special delivery form for commercially important adjuvants, by a saponin fraction of relatively high purity.

This is a collaborative project with Alberto Estrada of Animal Biotechnology Centre, Department of Animal and Poultry Science. Activities concerning this ongoing ADF Project are reported in more detail elsewhere.

16 b) Studies on Antioxidative Activities

Preliminary screening of over 15 Saskatchewan herbs, wild and cultivated, was conducted to date. Licorice root (*Glycyrrhiza lepidota*, *Glycyrrhiza glabra*, and *Glycyrrhiza uralensis*), roots of *Astragalus membranaceous* (Chinese milkvetch), *Althea officinalis* (marshmallow), *Arctium lappa* (burdock), and *Silybum marianum* (milk thistle) seed, are some of the species studied. The antioxidative activity, free-radical scavenging effect and reducing power of ethanolic extracts from leaves, roots, and seeds of three *Echinacea* species (*E. angustifolia*, *E. purpurea*, and *E. pallida*) were also

investigated. Ethanolic extracts displayed a mild antioxidative effect in meat model system and lesser effect in bulk oil systems.

This is an ongoing collaborative project with Ron Pegg of Saskatchewan Food Product Innovation Program, Department of Applied Microbiology and Food Science Activities concerning this ongoing ADF Project are reported in more detail elsewhere.

16 c) Studies on Anticancer Medicine

Main activities on this project included selection of wild plant species for the study and field collection of the plant material. Knowledge of Traditional Chinese Medicine (TCM) was used as a selection criteria in plant collection. Most collection took place in a 30 km radius around Saskatoon, some in the vicinity of Prince Albert and La Ronge. Dr. Yao Ge Huang, from the College of Chinese Agriculture Medicinal Materials, Jilin Agricultural University, who joined our team in March 1999 is a leading researcher on this project. Upon reviewing plant screening protocols for anticancer testing, including the protocol from United States National Cancer Institute (NCI), a tentative extraction protocol has been adopted. Four extracts (methanol, hexane, chloroform, water) were generated from every single plant and subjected for testing against three types of cancer cell lines.

This is a joint project with Robert Hickie of Department of Pharmacology, School of Medicine. Activities concerning this ongoing ADF Project are reported in more detail elsewhere.

OBJECTIVE 4: To establish communication and, where possible, collaboration with producers, government and research groups with common interest in herbs.

Achievements

17. Other Collaborative Projects and Miscellaneous Activities

Over the past 30 months the SHRP was involved in several research projects, one interprovincial project with Alberta and Manitoba addressing the need to identify superior seed sources of *Echinacea angustifolia*, and two other projects listed below.

17 a) Project on Herb Quality Management (AFIF)

The Herb Research Program conducted phytomedicinal quality testing of feverfew, echinacea, milk thistle, German chamomile and stinging nettle to date. This project's activities are complementary to the Herb Agronomy Research conducted by the SIDC in Outlook and Post-Harvest Processing Research conducted by the Agriculture and Bioresource Engineering Department, University of Saskatchewan. Detailed activities concerning this ongoing project are reported elsewhere.

17 b) Project on "Northern Vigour" Potential in Medicinal and Aromatic Plants (ADF)

This is a second year of a 3-year joint project with Karen Tanino, Department of Plant Sciences, and three US universities, UC Davis, Purdue and Oregon State in Corvallis. The objective of the project is to test the hypothesis that medicinal plants of superior quality could be grown at northern latitudes in Saskatchewan. The plant species included in the project are: catnip, valerian, calendula, echinacea, burdock and Chinese angelica. Detailed activities concerning this ongoing project are reported elsewhere.

17 c) Miscellaneous Activities

Collaboration with Saskatchewan Herb and Spice Association (SHSA): The communication with SHSA has intensified by more regular information exchange, updates on industry needs and how to best meet them. Several members of the Board of Directors of the SHSA have attended tour of the Herb Research Program facilities during PMAP Conference in March 1998. That was an excellent opportunity for them to get an insight into the scope of our research activities. Also, a study on optimizing harvest time for echinacea root was undertaken through communication and collaboration with SHSA.

Barl regularly provided research updates at the Annual Meetings of the SHSA and contributed to the SHSA Newsletter.

Collaboration with Saskatchewan Nutraceutical Network (SNN): Barl has established regular communicating with the SNN and its president Kelley Fitzpatrick. She reviewed the SNN Herb Industry Questionnaire aimed at assessing status and current and future needs of the growing number of Saskatchewan phytopharmaceutical and nutraceutical companies. Barl was an invited speaker for the SNN Seminar series and also accepted to be the member of the Scientific Advisory Committee of SNN.

Committee Work and Other Activities: Barl has been acting as a member of the national Expert Advisory Committee (EAC) on Complementary Medicines to Health Production Branch, Therapeutic Products Program in Ottawa, and on the Board of Directors of the Canadian Herb Society since 1998. She was the member of the Organizing Committee for the Prairie Medicinal and Aromatic Plants Conference held in Saskatoon in February 1998, and is presently also a member of the committee involved in organizing International Herb Conference to be held in Saskatoon, July 18-21, 2000.

D. Personnel

Name	Branka Barl
Category	Professional Research Associate
% Time	100% (full term)
Name	Donna Dunlop
Category	Research Assistant
% Time	100% (full term)
Name	Arin Katrusiak
Category	Research Assistant
% Time	100% (3 months)
Name	George Katselis
Category	Graduate Student
%Time	100% (2 months)
Name	Jasper Bintner
Category	Summer Student Assistant
% Time	100% (4 months)

Name	Demetris Papadakis
Category	Summer Student Assistant
% Time	100 % (4 months)

Name	Laura Cadrin
Category	Summer Student Assistant
% Time	50 % (4 months)

Name	Deana Dumont
Category	Casual Worker (Herb Plot)
% Time	40 % (4 months)

E. Equipment

A new high performance liquid chromatograph (HPLC) with diode array detector (Waters) was purchased to allow SHRP to conduct larger number of chromatographic assays and increase its research capabilities. Part of the cost was covered by this ADF grant.

F. Project Development Material

Refereed Journals:

Estrada, A., Katselis, G.S., Laarveld, B. and Barl, B. 1999. Isolation and evaluation of immunological adjuvant activities of saponins from *Polygala senega* L. Comparative Immunology, Microbiology and Infectious Diseases 23(1): 27-43

Amarowicz, R., Barl, B. and Pegg, R. 1999. Potential natural antioxidants from Saskatchewan indigenous plants. J. Food Lipids 6: 317-329.

Patent Granted or Pending

Estrada A., Barl B., Katselis G.S. and Laarveld, B. 1997. *Polygala senega* compositions and methods of use. United States Patent Application Serial No. 08/909.009.

Amarowicz, R., Barl, B. and Pegg, R.B. 1999. Composition extracted from plants used as antioxidant, United States Provisional Patent Application filed June 25, 1999.

Papers in Published Conference Proceedings and Abstracts:

Katselis, G., Estrada, A. and Barl, B. 1999. Chemical and immunological evaluation of saponins from *Polygala senega* L. In: 2nd International Conference on Saponins, September 6-8, Pulawy, Poland, p.1-6.

B. Barl, D. Dunlop and A. Katrusiak. 1999. Use of chemical markers for identification of species in the genus Echinacea. In: Proceedings of Soils & Crops '99 Conference, February 25 & 26, 1999, Saskatoon SK, p.257-262.

Barl, B., Gibson, N., Crerar, B., J. Shao and Sokhansanj, S. 1999. Feasibility of producing comfrey (*Symphytum* spp.) pellet as a feed supplement. In: Proceedings of Soils & Crops '99 Conference, February 25 & 26, 1999, Saskatoon SK, p. 414-419.

Barl, B., Amarowicz, R. and Pegg, R. 1999. Antioxidant activity of extracts obtained from selected species in the genus Echinacea. In: Abstracts of the Annual Meeting of Canadian Institute of Food Science and Technology (CIFST), June 5-9, 1999, Kelowna B.C., p. 75.

Barl, B. 1998. The potential for echinacea production in Western Canada. In: Proceedings of the Cropportunities IV Conference, July 21-22, University of Saskatchewan, Saskatoon, SK, p. 32-41, 1997

Katrusiak, A., Tanino, K. and Barl, B. 1998. Determination of flavonol glycosides and essential oil content in *Calendula officinalis* L. grown in Saskatchewan. In: Abstracts of Annual Meeting of Canadian Botanical Association, June 27-July 1st, Saskatoon, SK, p. 112

Conference Presentations:

B. Barl, D. Dunlop and A. Katrusiak. 1999. Species identification of echinacea seed and root using chemical markers. Herb Research Day/Richters Fourth Commercial Herb Growing Conference, Uxbridge, Ontario, October 22-24.

Huang, Y.G., Barl, B. and Ivanochko, G. 1999. Some non-timber forest products produced in Jilin Province, China. First International Conference on Non-Timber Forest Products in Cold Temperate and Boreal Forests, Kenora, Ontario, October 1-4.

B. Barl. 1998. Medicinal, Aromatic and Spice Crops-Prairie Perspective. 3rd Annual Herb Growers Conference, February 6-7, Burnaby, British Columbia.

B. Barl. 1998. The importance of quality in herb production and processing. Prairie Medicinal & Aromatic Plants Conference 1998, March 5-7, Saskatoon, Saskatchewan.

Barl, B., Dunlop, D. and Katselis, G. 1998. Overview of the Herb Research Program at the University of Saskatchewan. Prairie Medicinal & Aromatic Plants Conference 1998, March 5-7, Saskatoon, SK, p. 1 (Poster Session)

Barl, B. and Dunlop, D. 1998. Species authentication and content of marker compounds in three Echinacea species of commercial importance. In: Proceedings of the Prairie

Medicinal & Aromatic Plants Conference 1998, March 5-7, Saskatoon, SK, p.2 (Poster Session)

Other Publications:

Troesch, M., Funk, C. and Barl, B. August 1997. Silybum marianum (Milk Thistle) Infosheet, p.4

Troesch, M., Funk, C. and Barl, B. August 1997. Valeriana officinalis (Valerian) Infosheet, p.4

Barl, B. Conference Report: Prairie Medicinal & Aromatic Plants Conference 1998, March 5-7, Saskatoon. *In*: Herbal Times 3 (1A): 17.

Barl, B. 1999. News from the Saskatchewan Herb Research Program: Effect of harvest time on phytomedicinal quality of selected medicinal plants. SHSA Newsletter, March, p. 11-12.

Barl, B. 1998. News from the Saskatchewan Herb Research Program: Northern Vigour™ potential in medicinal and aromatic plants. SHSA Newsletter, September 1998, p.5-6.

Barl, B. 1997. News from the Saskatchewan Herb Research Program, SHSA Newsletter, December 1997, p.7-8.

Presentations

Over 25

G. SDAF Acknowledgment

Same as under F.

H. Expense Statement

Statement of expenditures and receipts has been submitted by the Office of Research Services, University of Saskatchewan.

APPENDICES

Appendix A: List of plants grown in the Herb Demonstration and Resource Garden, Department of Plant Sciences, University of Saskatchewan

HERB DEMONSTRATION AND RESOURCE GARDEN - August 1998

MEDICINAL PLANTS

Angelica (*Angelica archangelica*)
Arnica, American (*Arnica chamissonis*)
Balm, lemon (*Melissa officinalis*)
Burdock, great (*Arctium lappa*)
Calendula (*Calendula officinalis*)
Chamomile, German (*Matricaria recutita*)
Chamomile, Roman (*Anthemis nobilis*)
Chamomile, scentless (*Matricaria maritima*)
Clover, red (*Trifolium pratense)*
Comfrey, common (*Symphytum officinale*)
Comfrey, Russian (*Symphytum x uplandicum*)
Comfrey, Russian - Bocking No. 14 (*Symphytum x uplandicum*)
Comfrey, Russian - Bocking No. 4 (*Symphytum x uplandicum*)
Coneflower, narrowleaf (*Echinacea angustifolia*)
Coneflower, pale-purple (*Echinacea pallida*)
Coneflower, purple (*Echinacea purpurea*)
Coneflower, yellow purple (*Echinacea paradoxa*)
Dandelion (*Taraxacum officinale*)
Elecampane (*Inula helenium*)
Eleuthero; Siberian ginseng *(Eleutherococcus senticosus)*
Evening primrose, yello, wild (*Oenothera biennis*)
Feverfew (*Chrysanthemum parthenium*)
Horehound (*Marrubium vulgare*)
Licorice (*Glycyrrhiza glabra*)
Licorice, wild (*Glycyrrhiza lepidota*)
Licorice, Chinese; Gao-cao (*Glycyrrhiza uralensis*)
Lovage (*Levisticum officinale*)
Marshmallow (*Althaea officinalis*)
Milkvetch, Chinese (*Astragalus membranaceus*)
Motherwort (*Leonurus cardiaca*)
Motherwort, Chinese- Yi-mu-cao (*Leonurus artemisia*)
Mugwort (*Artemisia vulgaris*)
Mullein (*Verbascum thapsus*)
Pineapple weed (*Matricaria matricarioides*)
Plantain (*Plantago lanceolata*)
Plantain, common (*Plantago major*)
Rhubarb, Chinese/Turkish (*Rheum palmatum*)
Seneca snakeroot (*Polygala senega*)
Sorrel, sheep (*Rumex acetocella*)

MEDICINAL PLANTS (cont'd)

Speedwell (*Veronica officinalis*)
St. John's wort (*Hypericum perforatum*)
Stinging nettle (*Urtica dioica*)
Tea Tree (*Melaleuca alternifolia*)
Tibetan gentian (*Gentiana tibetica*)
Valerian (*Valerian officinalis*)
Vitex (*Vitex negundo heterophylla*)
Wormwood (*Artemisia absinthium*)
Wormwood, fringed; Prairie/pasture sage (*Artemisis frigida*)
Wormwood, Sweet Annie; Qing-guo (*Artemisia annua*)
Yarrow- white, yellow, cerise queen, wild (*Achillea millefolium*)

AROMATIC PLANTS

Anise-hyssop (*Agastache foeniculum*)
Bergamot, lemon (*Monarda citriodora*)
Bergamot, purple (*Monarda fistulosa*)
Bergamot, red (*Monarda didyma*)
Catnip (*Nepeta cataria*)
Hyssop (*Hyssopus officinalis*)
Lavender, Munstead (*Lavandula angustifolia 'Munstead'*)
Rue (*Ruta graveolens*)

CULINARY HERBS

Chives (*Allium tuberosum*)
Dill (*Anethum graveolens*)
Oregano (*Origanum spp.*)
Parsley, plainleaf/Italian (*Petroselinum crispum neapolitanum*)
Parsley, curled (*Petroselinum crispum crispum*)
Sorrel (*Rumex acetosa*)
Sorrel, French (*Rumex scutatus)*
Thyme, English (*Thymus vulgaris*)

PLANTS WITH INSECTICIDAL PROPERTIES

Pyrethrum (*Chrysanthemum cineriifolium*)
Rhubarb, cv's: MacDonald, Canada Red, Valentine, Honey Red German Wine, Irish Giant (*Rheum* spp.)
Tansy (*Tanacetum vulgare*)

SASKATCEWAN WILD FLOWERS

Alfalfa, blue (*Medicago spp.*)
Alumroot (*Heuchera richardsonii*)
Bedstraw, northern (*Galium boreale*)
Chicory, wild (*Cichorium intybus*)

SASKATCEWAN WILD FLOWERS (cont'd)

Fireweed (*Epilobium angustifolium*)
Gaillardia (*Gaillardia aristata)*
Heart-leaved Alexander (*Zizia aptera*)
Hellenium (*Hellenium montanum)*
Meadow blazing star (*Liatris liguslistylis*)
Meadow-rue (*Thalictrum venulosum*)
Prairie Coneflower (*Ratibida columnifera)*
Sainfoin (*Onobrychis viciaefolia*)
Silverweed (*Potentila anserina*)

Appendix B: List of analytical services provided to herb industry (August 1, 1999-January 31, 2000)

Source	Sample	Request	Analysis Performed
Northwest Venture Farms Coeur d'Alene, ID	St. John's Wort coarsely ground herb (2 samples)	quantitation of active ingredients	hypericin, pseudohypericin, rutin, hyperoside, quercitrin, quercetin content
John Stuart Hardisty	St. John's Wort aerial parts	quantitation of active ingredients	hypericin, pseudohypericin, rutin, hyperoside, quercitrin, quercetin content
Wanda Wolf Cutknife	St. John's Wort flowering tops	quantitation of active ingredients	hypericin, pseudohypericin
Wise Owl Herbs Co. Ltd. Unity	*E. angustifolia* root (3 samples)	quantitation of active ingredients	echinacoside, cynarin, chlorogenic acid, rutin and chicoric acid content
Henderson Qualico Echinacea Quesnel	*Echinacea* spp. seed	species identification	TLC/HPLC fingerprint
Wise Owl Herbs Co. Ltd. Unity	*E. angustifolia* Spray dried extract (4 samples)	quantitation of active ingredients	echinacoside, cynarin, chlorogenic acid, rutin and chicoric acid content
Sherry Jurke Maidstone	*Echinacea* spp. seed	species identification	TLC/HPLC fingerprint
Sherry Jurke Maidstone	*E. angustifolia* root (2 samples)	quantitation of active ingredients	echinacoside, cynarin, chlorogenic acid, rutin and chicoric acid content
Rick Alsager Maidstone	*Echinacea* spp. seed	species identification	TLC/HPLC fingerprint
Rick Alsager Maidstone	*E. angustifolia* root	quantitation of active ingredients	echinacoside, cynarin, chlorogenic acid, rutin and chicoric acid content
Bowen Island Botanicals Bowen Island	St. John's Wort tincture (3 samples)	quantitation of active ingredients	hypericin, pseudohypericin
Bowen Island Botanicals Bowen Island	St. John's Wort ground aerial parts (3 samples)	quantitation of active ingredients	hypericin, pseudohypericin, rutin, hyperoside, quercitrin, quercetin content

Appendix B: (cont.)

Source	Sample	Request	Analysis Performed
Bowen Island Botanicals Bowen Island	*E. purpurea* root	quantitation of active ingredients	chicoric acid, chlorogenic acid, cynarin, echinacoside and rutin content
Bowen Island Botanicals Bowen Island	*E. angustifolia* root	quantitation of active ingredients	echinacoside, cynarin, chlorogenic acid, rutin and chicoric acid content
Bowen Island Botanicals Bowen Island	*Echinacea* spp. ground complex	quantitation of active ingredients	echinacoside, cynarin, chlorogenic acid, rutin and chicoric acid content
Bowen Island Botanicals Bowen Island	*E. purpurea* coarsely ground herb	quantitation of active ingredients	chicoric acid, chlorogenic acid, cynarin, echinacoside and rutin content
Bowen Island Botanicals Bowen Island	*Echinacea* spp. complex tincture (2 samples)	quantitation of active ingredients	echinacoside, cynarin, chlorogenic acid, rutin and chicoric acid content
Bowen Island Botanicals Bowen Island	*E. purpurea* tincture (2 samples)	quantitation of active ingredients	chicoric acid, chlorogenic acid, cynarin, echinacoside and rutin content
Wise Owl Herbs Co. Ltd. Unity	*E. angustifolia* powdered extract (2 samples)	quantitation of active ingredients	echinacoside, cynarin, chlorogenic acid, rutin and chicoric acid content
John Kort Indian Head	*E. angustifolia* root	quantitation of active ingredients	echinacoside, cynarin, chlorogenic acid, rutin and chicoric acid content
Wise Owl Herbs Co. Ltd. Unity	*E. angustifolia* ground root	quantitation of active ingredients	echinacoside, cynarin, chlorogenic acid, rutin and chicoric acid content
Wise Owl Herbs Co. Ltd. Unity	*E. angustifolia* spent ground root	quantitation of active ingredients	echinacoside, cynarin, chlorogenic acid, rutin and chicoric acid content
Wise Owl Herbs Co. Ltd. Unity	*E. angustifolia* spent ground root (washed)	quantitation of active ingredients	echinacoside, cynarin, chlorogenic acid, rutin and chicoric acid content

Appendix B: (cont.)

Source	Sample	Request	Analysis Performed
Wise Owl Herbs Co. Ltd. Unity	*E. angustifolia* extract	quantitation of active ingredients	echinacoside, cynarin, chlorogenic acid, rutin and chicoric acid content
Wise Owl Herbs Co. Ltd. Unity	*E. angustifolia* extract SD1/walls	quantitation of active ingredients	echinacoside, cynarin, chlorogenic acid, rutin and chicoric acid content
Nadine Froehlich Marquis	*E. angustifolia* root	species identification	HPLC fingerprint
Nadine Froehlich Marquis	*E. angustifolia* root	quantitation of active ingredients	echinacoside, cynarin, chlorogenic acid, rutin and chicoric acid content
Cornercopia Farms Wynndel	*Echinacea* spp. seed	species identification	TLC/HPLC fingerprint
Mark Martin Glenboro	*E. angustifolia* root	species identification	HPLC fingerprint
Mark Martin Glenboro	*E. angustifolia* root	quantitation of active ingredients	echinacoside, cynarin, chlorogenic acid, rutin and chicoric acid content
J. Huyghebuert Regina	*Echinacea* spp. seed (2 samples)	species identification	TLC/HPLC fingerprint
Manitoba Agriculture Carman	*E. angustifolia* root	quantitation of active ingredients	echinacoside, cynarin, chlorogenic acid, rutin and chicoric acid content
Manitoba Agriculture Carman	Valerian root	quantitation of active ingredients	valerenic acid content
Sikora Herb Farms Warspite	*E. angustifolia* root	quantitation of active ingredients	echinacoside, cynarin, chlorogenic acid, rutin and chicoric acid content
Wise Owl Herbs Co. Ltd. Unity	*E. angustifolia* commercial extract	quantitation of active ingredients	echinacoside, cynarin, chlorogenic acid, rutin and chicoric acid content
Don Perrault Humboldt	*Echinacea* spp. seed	species identification	TLC/HPLC fingerprint
Treasure Valley Markets Cadillac	*Echinacea* spp. root	quantitation of active ingredients	echinacoside, cynarin, chlorogenic acid, rutin and chicoric acid content

Appendix B: (cont.)

Source	Sample	Request	Analysis Performed
Blue Hills Herb Farms Regina	*E. angustifolia* root	quantitation of active ingredients	echinacoside, cynarin, chlorogenic acid, rutin and chicoric acid content
Blue Hills Herb Farms Regina	*Echinacea* spp. seed	species identification	TLC/HPLC fingerprint
Frank Mowers Moose Jaw	*Echinacea* spp. seed	species identification	TLC/HPLC fingerprint identification
Myron Erikson Cabri	*Echinacea* spp. seed	species identification	TLC/HPLC fingerprint
Judy Greeno New Dayton	*E. angustifolia* ground root	quantitation of active ingredients	echinacoside, cynarin, chlorogenic acid, rutin and chicoric acid content
Wilfred Schafer Richmond	*E. angustifolia* root	species identification	HPLC fingerprint
Richard Ens Nipawin	*E. angustifolia* root	quantitation of active ingredients	echinacoside, cynarin, chlorogenic acid, rutin and chicoric acid content
Dean Klippenstein Moose Jaw	*Echinacea* spp. seed (2 samples)	species identification	TLC/HPLC fingerprint
Chemica West Saskatoon	Cilantro (3 samples)	analysis of essential oil	GC analysis of linalool content
Chemica West Saskatoon	Cilantro (12 samples)	analysis of essential oil	GC analysis of linalool and decanal content
Chemica West Saskatoon	Decanal standard	analysis of standard	GC analysis of decanal content
NorSask Botanicals Tisdale	Valerian root (2 samples)	quantitation of active ingredients	valerenic acid content
Schafer's Health Centre Unity	*E. angustifolia* extract	quantitation of active ingredients	echinacoside, cynarin, chlorogenic acid, rutin and chicoric acid content
University of Guelph Guelph	Commercial feverfew tablets, capsules and soft gel caplets (12 samples)	quantitation of active ingredients	parthenolide content